CONTRIBUTION OF SPIDER RESOURCE USE TO ECOSYSTEM NUTRIENT FLOW AND FUNCTION

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Abstract: At the interface of the interactions between organisms and their environments, nutrient cycling dynamics significantly shape ecosystems. Ecological stoichiometry focuses on the availability and balance of elements through ecosystems, however, elemental analyses can oversimplify the animal nutrition and physiology components of biochemical flow through ecosystems. The study of consumer-driven nutrient recycling traces the fate of ingested elements and other nutrients through consumer excretive processes and the role of nutrient deposition in ecosystem nutrient flow. But, the study of nutrient recycling has been largely limited to the interactions between primary producers and herbivores, generally in aquatic or pelagic systems. Research of predators could therefore broaden applications of nutrient recycling theory. The goal for my dissertation was to explore the mechanisms through which spiders influence ecosystem function. The scope of my research spanned the investigation of digestive physiology and excretion up to the broader assessment of how predators contribute to soil microbial communities and plant growth. I also sought to resolve applications of different nutrient frameworks, from elements to macronutrients. I first tested how variation in prey nutrient content affected the flow of nutrients through different pathways of spider physiology. Next, I tested whether prey exoskeleton influenced the flow of nutrient through web-building spiders as carcass deposition, assimilation, digestive metabolism, and excretion. While my first two dissertation chapters examined the forms of nutrient inputs by spiders, my third chapter examined how each of the nutrient inputs affects the flow of nutrients to soil communities and plant growth. Finally, I tested for spatial effects of predator nutrient supplementation to soil and plant systems. My results discern the nutrient cycling roles of spiders as opportunistic, generalist predators and predominantly indirect contributors to soil and plant processes. That is, while I found strong evidence that spiders assimilate more nutrients from less-chitinous prey and grow more on lipid-biased diets, I also found that spiders assimilated most available nutrients and there was not a difference in nutrients entering the soil interface based on prey composition. But, the labile nutrients comprising spider excreta were important for increasing soil decomposition of more complex nutrients and this indirectly bolstered plant growth and nutrients. Future study will be necessary for further elucidating the role of terrestrial predators in consumer-driven nutrient cycling. Finally, the inclusion of predators in future testing of consumer-driven nutrient cycling perspectives offers a broad opportunity to further advance the understanding of ecosystem nutrient flow and function.

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General Introduction

At the interface of the interactions between organisms and their environments, biogeochemical dynamics significantly shape ecosystems. Nutrients in the abiotic environment are converted into living tissue by primary producers and transferred through food webs through trophic interactions. Ultimately, nutrients in the biotic system return to the environment as excreta or dead tissue of organisms. Developing a mechanistic understanding of how changes in food webs affect the function of ecosystems is important for predicting how ecosystems will change in response to growing human communities or climate change.

Ecologists have traditionally united abiotic and biotic factors in the study of ecosystem dynamics by following the flow of energy and nutrients. Classical ecologists documented energetic productivity within communities and trophic levels (Elton 1927, Lindeman 1942). From a different perspective, the foundational ecology paper by Redfield (1958) unraveled the trajectory and ratios of elemental resources through the marine biochemical cycle, laying the framework for the field of ecological stoichiometry. Ecological stoichiometry focuses on the availability and balance of elements through ecosystems (Sterner and Elser 2002). However, elemental analyses can oversimplify the animal nutrition and physiology components of biochemical flow through ecosystems (Anderson et al. 2004, Anderson and Pond 2000). For example, the sensory systems of consumers are typically

associated with molecular compounds, rather than elements (Raubenheimer and Simpson 2004). Further, although consumers consume complex molecules of carbon and nitrogen (e.g. lipids, proteins, and carbohydrates), measurements of these elements do not differentiate between digestible and indigestible nutrients, such as the carbon contained in lipid versus that of chitin (Wilder and Eubanks 2010). Thus, development of inferences pertaining to ecosystem nutrient flow and function will require integration of these frameworks with stoichiometry used at the interface of abiotic and biotic interactions and nutritional frameworks better suited for transfers of nutrients during biotic interactions.

Predators contribute to ecosystem function and nutrient cycling through complex trophic interactions. Notable examples of predator impacts within ecosystems are trophic cascades, nutrient consumption, nutrient translocation, and indirect effects of predation (Schmitz et al. 2010). However, the focus of ecological stoichiometry and ecosystem ecology has generally been on herbivores and omnivores. Previous research in ecological stoichiometry demonstrated that predators have higher relative nitrogen body content than herbivores (Fagan et al. 2002, Dennno and Fagan 2003), but whether predators are more nitrogen-limited or not has received criticism (Wilder and Eubanks 2010). Further, study of nutrient recycling has largely been limited to the interactions between primary producers and herbivores (Elser and Urabe 1999). To construct a more broadly applicable understanding of ecosystem nutrient flow and function, research of predators could contribute potentially transformative mechanisms.

The overall goal for my dissertation was to examine the mechanisms through which spiders influence ecosystem function. The scope of my research spanned investigation of the physiology of digestion and excretion up to the broader assessment of how carnivores

contribute to soil microbial communities and plant growth. My dissertation has been divided into four research objectives that progressively increase in biological scale from individual physiology to ecosystems.

In my first dissertation chapter, I tested how variation in prey nutrient content (i.e., the ratio of lipid:protein in prey) affected the flow of nutrients through different pathways of spider physiology including assimilation, egestion, respiration, and excretion. This study was conducted on a wolf spider, Hogna carolinensis. Jensen et al. (2011) found that wolf spiders grew larger carapaces on more protein-biased diets and weighed more on more lipid-biased diets. Further, in this chapter I explore the efficacy of applying the traditional nitrogen conversion factor as a surrogate for estimation of protein content (protein= nitrogen x 6.25; Jones 1941). Finally, I tested the stoichiometric theory prediction that the relative amounts of nutrients recycled by organisms are a function of mass balance between consumer and resource (i.e. consumer-driven nutrient cycling; Elser and Urabe 1999, Vanni et al. 2002, Hood et al. 2005).

Previous evaluation of ecological stoichiometry predictions for consumer-driven nutrient cycling has been primarily applied in the contexts of aquatic and pelagic systems. However, comparative perspectives using terrestrial predators have not yet been developed. Terrestrial arthropod prey vary in their exoskeleton content and spiders vary in their feeding strategies, from wandering ambushers to web-builders. Hence, my second chapter compares the flow of nutrients through the bodies of web-building spiders. Only a limited number of studies have assessed the standard, feeding, and other metabolic rates of arachnids (e.g. (Young and Block 1980, Jensen et al. 2010, Nespolo et al. 2011). The exoskeleton content of arthropods varies greatly (9 – 60% of dry mass; Lease and Wolf 2010), however, the impact

on predator assimilation, digestive metabolism, and excretion is not yet well understood. In this chapter, I predicted that spiders consuming prey with a greater amount of exoskeleton would extract fewer resources from their prey, leaving a greater proportion of nutrients in the prey remains. I also predicted that digestive metabolic cost (i.e. Specific Dynamic Action) would increase with exoskeleton content due to increased cost of puncturing and sucking nutrients from prey. However, mechanisms linking spider nutrient consumption and deposition to ecosystem nutrient flow remain an unexplored frontier.

While my initial two dissertation chapters examine the forms of nutrient inputs provided by spiders (e.g. excreta and egesta), my third dissertation chapter examined how each of these types of nutrient inputs affects the flow of nutrients to the soil communities and plant growth. The goal for this chapter was to test how variation in prey nutrient content affects spiders and the contribution of spiders and prey to soil nutrient dynamics, including soil respiration and subsequent plant growth. The soil layer serves as a primary biogeochemical interface in terrestrial ecosystems upon which many resources are extracted and deposited. Labile organic nutrients (i.e. excreta) are crucial primers of soil microbial processing of more complex organic compounds (Hawlena et al. 2012). In turn, recycling of nutrients by soil communities are critical drivers of aboveground primary production and I predicted that input of spider labile nutrients would most greatly spur plant growth on lowest nutrient soils. Although the contribution of spiders to nutrient flow beneath the web could be an important driver of local soil and plant processes, another important consideration is that areas near webs could be nutrient hotspots relative to locations further away and that spiders could be contributing to nutrient distribution heterogeneity.

My final chapter tested for spatial effects of predator nutrient supplementation to soil and plant systems. I hypothesized that soils taken from beneath the spiders' webs would have the highest soil nutrients, soil respiration rates, plant growth and foliar nutrient levels relative to samples taken at the periphery of spider webs, and in areas away from the spider webs. Carnivores can mediate the spatial distribution of carcasses (Bump et al. 2009) and excrement (Romero et al. 2006), which can increase heterogeneity in soil and plant production. Further, spider webs entrap nutrients at highly localized scales and can increase soil productivity (Hodkinson et al. 2011). In this concluding component of my dissertation, I investigated soil respiration and plant growth below spider webs versus away from the web.

From the initial level of individual consumer physiology to ultimately ecological community-levels, my dissertation examined mechanisms by which spiders contribute to ecosystem nutrient flow. I predicted that spider nutrient consumption and deposition would be important drivers of soil and plant community processes. I also sought to resolve applications of different nutrient perspectives, from elements to macronutrients. My experimental findings could broaden the understanding of ecosystem nutrient flow, with particular recognition on the novelty of carnivore ecology.

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CHAPTER I

Mismatch Predators Buffer the Effects of Variation in Prey Nutrient Content for Nutrient Deposition

Abstract

Predator feeding behavior and digestion regulate the flow of nutrients through ecosystems by determining the fate of prey nutrients. Most predators feed on a diversity of prey items, which differ widely in traits including their nutrient content. Yet, relatively little is known of the mechanisms through which variation in prey nutrient content affects the form by which nutrients are deposited into the environment. The overall goal of this study was to test how variation in the nutrient content of prey affected the fate of nutrients following predation by an arthropod carnivore, the Carolina wolf spider (*Hogna carolinensis*). We manipulated the macronutrient content of prey by varying the diet on which crickets were fed to produce prey treatments that differed in lipid and protein content. Nutrients were measured as both macronutrients and elements in prey and elements in excreta. We found that there was no effect of diet treatment on the amount of elements or macronutrients in prey carcasses and excreta despite significant variation in the nutrient content of those prey. This is in contrast to studies of some aquatic systems where mass balance by consumers results in variation in excreta content depending on the nutrient content of food. Wolf spiders assimilated the majority of prey nutrients and deposited relatively small and similar amounts of nutrients following feeding. Hence, while prey can vary

widely in nutrient content, our findings suggest that this variation has little effect on the amounts of nutrients deposited by predators.

Introduction

Predators regulate resource transfer, trophic interactions, and ecosystem function, including nutrient deposition and mineralization (Schmitz 2007, Hawlena and Schmitz 2010, Schmitz et al. 2010, Hawlena et al. 2012). For example, bears can transfer significant amounts of nutrients from salmon to surrounding terrestrial habitats (Hilderbrand et al. 1999), excreta of seabirds can be a major source of nutrients on islands (Anderson and Polis 1999), and predation risk by spiders can shift the nutrient composition of grasshoppers and, ultimately, decomposition processes (Hawlena et al. 2012). However, while studies have shown that predators affect nutrient flows in ecosystems, less remains known about the mechanisms through which predators have these effects.

Predators regulate biogeochemical processes in ecosystems by determining the fate of prey nutrients. Digestible nutrients such as lipid and protein are ingested, digested, and either assimilated, excreted or respired. Other parts of prey such as structural compounds in the skeleton are either discarded following feeding or deposited in feces. Some of these forms of nutrients may be more readily utilized by microbes, decomposers and primary producers (e.g., excreta) while others may take years to decompose (e.g., prey exoskeleton; Seastedt and Tate 1981).

One factor that could affect how predators regulate the fate of consumed nutrients is the chemical composition of their prey. Prey vary widely in their chemical composition both within and among species (e.g., 5 – 30 % lipid and 20 – 80 % protein; Fagan et al. 2002, Raubenheimer et al. 2007, Lease and Wolf 2010, Wilder et al. 2013). Previous studies of aquatic systems have shown that mass balance can be used to predict the nutrient content of excreta when consumers feed on different foods (Sterner 1990, Elser and Urabe 1999, Sterner and Elser 2002). For example, consuming food with higher N:P than that found in a consumer's body can result in the production of excreta with higher N content (Sterner 1990, Sterner and Elser 2002). While

digestive processing of prey is general to all predators, relatively little is known about its importance and how this process varies when feeding on different prey (Croll et al. 2005, Persson and Svensson 2006, Romero et al. 2006, Sin et al. 2008, Gharajehdaghipour et al. 2016). Spiders are good candidates for studying how digestive processes of predators affect nutrient deposition. First, spiders feed on an estimated 400-800 million tons of prey per year, which likely translates into a significant amount of nutrients deposited from these prey (Nyffeler and Birkhofer 2017). Second, spiders feed using extraoral digestion, which is a process used by the majority of arthropod predators and allows for easier quantification of digestible versus indigestible components of prey (Cohen 1995, Wilder and Eubanks 2010, Wilder et al. 2013). Finally, this work can build on a significant literature base that has examined the nutritional ecology of spiders and the consequences of prey nutrient content for spider physiology and growth (Wilder 2011, Toft 2013). For example, Jensen et al. (2011a) found that wolf spiders grew larger carapaces on more protein-biased diets and weighed more on more lipid-biased diets and that spiders will regulate their lipid and protein intake when possible (Jensen et al. 2011b). Understanding how prey nutrient content affects the quantity and form through which those nutrients are deposited in the environment will complement previous work on how diet affects predator growth and behavior (Wilder 2011, Jensen et al. 2011a, b).

The overall goal of this study was to test how variation in the nutrient content of prey affected the fate of nutrients following predation (Figure 1). We produced four prey types that differ in lipid and protein content by feeding cricket prey with varying diets. Nutrients were measured as both macronutrients and elements in prey and prey carcasses, and elements in excreta. Although prey macronutrients are often estimated from conversion factors based on elements (e.g. protein= nitrogen x 6.25; Jones 1941), these conversions do not differentiate between digestible and indigestible forms, such as protein versus N-rich exoskeleton (Wilder and Eubanks 2010). We hypothesized that spiders would ingest and excrete nutrients in proportions

similar to their availability in their prey. For example, we predicted spiders consuming prey with higher protein content would ingest more protein and excrete more nitrogen than spiders fed prey with higher lipid and lower protein.

Spiders were starved for 10 days prior to trials to standardize hunger level. Extensive evidence suggests that spiders including wolf spiders (Nyffeler and Benz 1988, Wise 1993) regularly experience such food deprivation in nature (reviewed in Wise 1993, 2006). For example, field-collected wolf spiders did not differ in body condition from laboratory conspecifics that were completely deprived of food for 3 months (Wilder and Rypstra 2008). Another field study estimated that two spider species experienced average starvation periods of 4-8 days (Bilde and Toft 1998).

Methods

Study Animals: Spiders, like the majority of arthropod predators, feed using extra-oral digestion (Cohen 1995). Nutrients are liquefied, filtered through the mouth and pharynx, digested, assimilated, respired or excreted as nitrogenous by-products (Foelix 2011). Spiders use digestive enzymes to liquefy edible nutrients (e.g., protein and lipid) and discard prey carcasses following feeding (Foelix 2011). Prey carcasses largely consist of indigestible exoskeleton (Foelix 2011).

For this study, fifty-eight mature female Carolina wolf spiders (*Hogna carolinensis*) were collected from local fields near Oklahoma State University (Stillwater, OK) in summer 2015. From the field, spiders were transferred to laboratory housing of 16oz plastic deli cups and were fed two, half-inch sized crickets (*Acheta domesticus*; purchased from Fluker Farms, Louisiana) twice per week. Crickets used for maintenance feeding were raised in the laboratory on dog food (Rachel Ray Nutrish). A water-soaked cotton ball and paper-towel bedding were changed twice per week. Wolf spiders and crickets were maintained on a constant 25 ± 1 ^{o}C and 10D:14L light regime.

Cricket Diets and Feeding Trials: We manipulated the nutrient content of crickets by feeding them different diets varying in carbohydrate, lipid and protein content. Crickets were maintained on specific diets for at least one week before being used as prey. Crickets were maintained on diets varying in protein, lipid, and carbohydrate (Protein:Lipid:Carbohydrate) at 100:0:0, 80:10:10, 60:20:20, and 10:45:45 percent (see Table 1 for the composition of the diets). Crickets were provided water ad libitum, which was replaced at least twice weekly. Periodically, we collected crickets from each diet to analyze their nutrient content. Prior to analysis of cricket bodies for nutrient content, representative crickets from each treatment were killed via chilling and stored in a freezer, then dried at 60 ^{O}C for 24 hours and weighed.

To standardize feeding level in the trials (Wilder and Rypstra 2008), each wolf spider was starved for 10 days, assigned to a cricket diet treatment, and placed in a 24oz Glasslock Weangreen glass container with a glass fiber substrate. The glass fiber filter used as a substrate in the arenas had the texture of paper and provided a surface for collecting spider excreta that contained no carbon or nitrogen itself. To reduce experimenter bias, spiders were assigned to treatments using a random number generator. A control chamber containing filter paper but no spider was also run alongside each cohort of spiders to test if handling of the paper resulted in C or N contamination, which it did not. To intersperse potentially confounding environmental effects, spider cohorts were rotated within the shelving unit once a week. Wolf spiders and prey were maintained in a constant temperature room at 25 ± 1 ^{O}C and 10D:14L light regime.

The wolf spiders were fed two crickets from their assigned treatment group, twice per week, for two weeks. During these feeding trials, prey remains, silk, and excreta were collected. Cricket remains were collected from the spider chambers after 24 hours using alcohol-sterilized tweezers, deposited in centrifuge tubes, and frozen. Spiders typically finished feeding on prey within 6 hours. Silk was removed from the spider containers weekly by wrapping it around a pair of alcohol-sterilized tweezers, depositing it in centrifuge tubes, and placing them in the freezer.

Several measures of excreta, including total excreta area, individual excreta mass, and density of excreta per unit area, were collected. An Olympus Stylus Tough TG-3 camera was used to photograph the experimental chambers weekly for digitization of the area (cm^2) of spider excreta using ImageJ 1.48v (National Institutes of Health, USA). Excreta tended to spread outward from the point of deposition as it dried. ImageJ was used to trace around the perimeter of each excreta. Counts of all excreta were also calculated. A hole-puncher was used to cut reference (i.e., glass filter only) and filter with excreta samples after pictures were taken. Excreta-free cutouts from spider containers served as within-treatment references. The masses of glass filter paper containing wolf spider excreta were deducted from the mass of similarly sized blank glass

filter paper. Then, the density (mass of excreta per unit area of paper) was multiplied by the total digitized area of excreta and multiplied by the proportion of nitrogen (see below) to estimate the mass of excreted nitrogen.

Nutrient Analyses: We froze the cricket remains, silk, and excreta following collection. Crickets and remains of crickets following feeding were randomly split between macronutrient and elemental analyses. All silk and excreta were run in elemental analysis. We measured lipid content as the difference in mass before and after sequential soaking and extraction in chloroform over the course of three days (Wilder et al. 2013). Protein content was determined in triplicates using the Bradford Assay on lean, ground samples (Wilder et al. 2013). Although we manipulated the amount of carbohydrates present in cricket diets, nutrient analysis of cricket body composition did not measure carbohydrates as they are typically present in arthropods at low concentrations (Raubenheimer and Rothman 2013). Crickets, like other omnivores, either metabolize or convert ingested carbohydrate to lipid reserves and do not use carbohydrates as a storage molecule in the body (Klowden 2013). Crickets, predation remains, silk, and spider excreta were evaluated for carbon and nitrogen content. The percentages present were quantified from combustion in an elemental analyzer (Elementar Americas, Inc., Mt. Laurel, New Jersey).

Nutrient ingestion was estimated by first calculating the expected nutrient content of prey fed to spiders using the wet mass of the prey and data from control prey items (i.e., linear regressions of wet mass versus nutrients). The nutrient content of prey remains was then deducted from the initial amount of nutrients present in the prey fed to the spiders to calculate the amount of nutrients ingested.

Statistical Analysis: We tested if variation in the macronutrient content of prey (4 treatments) affected amounts (mg/ 100 mg prey) of resources ingested, egested, excreted, and silk produced by the wolf spiders. Multivariate analysis of variance (MANOVA) was first used to determine if

there was an overall effect of the diet treatments on multiple responses. Following significant MANOVA effects, individual ANOVAs were run for each response variable. Statistics were assessed using JMP 12 software package (SAS Institute, Cary, NC, USA).

Results

Prey Content: We found significant differences in the lipid ($F_{3,51}$= 20.67, $p < 0.0001$) and protein ($F_{3,51}$= 25.84, $p < 0.0001$) content of crickets fed the different experimental diets, which confirmed the effectiveness of our diet manipulation treatments (Figure 2A). Crickets fed the highest protein food had the highest protein in their body and crickets fed the highest carbohydrate and lipid food had the highest lipid content in their bodies. Additionally, elemental analysis showed significant differences in nitrogen ($F_{3,54}$= 41.00, $p < 0.0001$) and carbon ($F_{3,54}$= 16.76, $p < 0.0001$; Figure 2B) among cricket treatments. Carbon content of crickets was higher in the higher carbohydrate and lipid diets, while nitrogen content was greatest in the higher protein treatments. The mean wet mass of crickets fed to spiders was highest in the 60:20:20 treatment, lowest in the 100:0:0 treatment and intermediate in the other treatments ($F_{3,54}$= 4.28, p= 0.009). Yet, the dry mass of crickets fed to spiders did not differ significantly among the diet treatments ($F_{3,50}$= 2.68, p=0.06), which suggests that crickets differed in water content but not total amount of nutrients provided to spiders.

Wolf Spiders Mass: The initial wet mass of spiders before receiving their experimental diets did not differ among treatments ($F_{3,55}$= 1.01, p= 0.40). Further, the mean wet masses of spiders on prey treatments did not differ after the first week ($F_{3,53}$= 2.35, p= 0.08) or final week of feeding ($F_{3,51}$= 1.52, p= 0.22). The mean change in mass between start to completion of the feeding trials did not differ by treatment ($F_{3,52}$= 0.35, p= 0.79).

Ingestion: From the MANOVA, we found a significant effect of prey treatment on the fates of nutrients ($F_{12,64}$= 15.36, $p < 0.0001$). We found that spiders fed higher lipid prey ingested more lipid ($F_{3,27}$= 137.28, $p < 0.0001$) and spiders fed higher protein prey ingested more protein ($F_{3,27}$= 16.07, $p <0.0001$; Figure 3A). The MANOVA also indicated that prey treatment affected elemental fates ($F_{12,106}$= 71.52, $p < 0.0001$). There were differences in nitrogen ($F_{3,43}$= 79.80, p <

0.0001) and carbon ingestion ($F_{3,43}$= 8.30, p= 0.0002;Figure 3B). The ingestion of carbon by spiders was greatest when fed high lipid prey and nitrogen ingestion was highest when fed higher protein prey.

Prey Remains: There were no effects of diet treatments on lipid ($F_{3,27}$= 0.67, p= 0.58) or protein ($F_{3,27}$= 0.48, p= 0.70; Figure 3A) remaining in the prey carcass. There was very little lipid and protein remaining in the carcasses when they were discarded by the spiders (e.g., typically less than 5% of the amount present in whole prey; Figure 1). Similarly, we did not detect a difference in the nitrogen ($F_{3,43}$= 2.19, p= 0.10) nor in carbon content of prey carcasses between the treatments ($F_{3,43}$= 1.43, p= 0.25; Figure 3B).

Silk: The quantity and composition of silk produced by wolf spiders did not differ by prey treatment. Overall, silk production was low, as might be expected for wandering spiders (Figure 1). The mass of silk produced by spiders did not differ by prey diet treatments (mean= 0.62 ± 0.13mg; $F_{3,39}$= 1.05, p= 0.38). Proportions of nitrogen (mean= 10.2 ± 0.53mg 100mg-1 silk) and carbon (34.9 ± 1.37 mg 100mg-1 silk) in silk did not differ between the prey treatments ($F_{3,17}$= 0.65, p= 0.59 and $F_{3,21}$= 0.02, p= 0.99, respectively).

Excretion: The content and quantity of nutrients in the wolf spider excreta did not differ by prey diet (Figure 1, Figure 4A and B). The number of excreta produced (mean= 12.36 ± 0.98 excreta per spider) did not differ between prey treatments ($F_{3,46}$= 1.58, p= 0.21), but was linearly related to the area of excreta ($F_{1,49}$= 9.01, p= 0.004, R2= 0.16). Regardless of prey diet, wolf spider nitrogen and carbon content within spider excreta were not significantly different among treatments ($F_{3,30}$= 2.06, p= 0.13 and $F_{3,30}$= 0.69, p= 0.56, respectively). The data for elemental content of excreta included 3 data points with much higher nitrogen and carbon content than the rest of the data set, which contributed to higher SE in the data. If these 3 data points are excluded,

there is still no significant effect of treatment on nitrogen and carbon content of excreta ($F_{3,27}$= 1.51, p= 0.23 and $F_{3,27}$= 0.12, p= 0.94, respectively).

Discussion

In nature, prey vary widely in elemental and macronutrient content (Fagan et al. 2002, Wilder et al. 2013). Our results show that predator assimilation buffers the effects of variation in prey nutrient content for nutrient deposition following predation. Regardless of the initial nutrient content of prey, spiders deposited the same amounts of C and N in prey carcasses, silk, and excreta (Figure 1). We found that wolf spiders on higher protein diets ingested higher protein and nitrogen than wolf spiders on higher lipid diets. But, there was no effect of prey nutrient content on the amount of nutrients or elements in prey carcasses and excreta, and both of these products comprised a small proportion of total prey nutrients (Figure 1). These results highlight the value of integrating data on digestive physiology into predictions for how predators or other consumers might influence nutrient cycling.

Spiders have often been hypothesized to be food-limited in nature and, hence, may have faced strong selection for efficient extraction and use of nutrients (reviewed in Wise 1993, 2006). In the current study, most of the macronutrients in the prey were ingested by predators (83-88%), rather than deposited in the prey remains. In addition, excreta production was relatively low. We estimate that spiders would immediately return only approximately 16-19% of total prey nitrogen to the patch in which they reside, while the rest (81-85%) would be assimilated. The high relative magnitude of these assimilation estimates is corroborated by other studies of resource assimilation efficiency in arthropod carnivores (81.4% in the wolf spider Pardosa lugubris; Edgar 1971 and 83-90% in damselflies; Lawton 1970). Furthermore, a large proportion of the nutrients deposited by the wolf spiders was in the form of exoskeleton (i.e. prey carcasses), which can take years to decompose (Seastedt and Tate 1981). The food-limited conditions used in our study likely reflect conditions experienced by many spiders and other predators in nature (Wise 1993). Yet under high feeding levels, some spiders have been observed to engage in superfluous killing (i.e., killing but not eating prey) or selective extraction of nutrients from prey (Riechert and

Maupin 1998, Mayntz et al. 2005). Further work is needed to examine how these patterns of nutrient extraction and deposition might differ under higher feeding levels, such as predators feeding on agricultural pests or during outbreaks of prey.

Our results show that combining macronutrient and elemental approaches can help better predict nutrient fates during trophic interactions. Spiders do not consume entire prey items. Rather, they liquefy and ingest soft tissues of prey and discard the indigestible parts (i.e., exoskeleton). As demonstrated in our results, spiders are very efficient at extracting nutrients from prey and discarded prey remains are almost entirely composed of exoskeleton. While exoskeleton has a large amount of C-rich chitin, it can also contain a large amount of N due to cross-linked proteins that are bound in the chitin matrix and, hence, inaccessible to both spiders and our soft-tissue protein assay (Klowden 2013). If we had estimated protein using the standard 6.25 conversion factor, these estimates would have been approximately 6-15% greater than that of our estimates using the Bradford protein assay, similar to what has been observed in previous research of crickets and other arthropods (Finke 2007). Analysis of elements alone cannot distinguish between N in the soft tissues of prey and N in the indigestible exoskeleton. Yet, the combination of macronutrient and elemental approaches could be used to partition N in prey into protein-N versus non-protein-N, which can then be used to better predict the fate of prey nutrients (e.g., ingested versus egested). Such distinctions may be especially important when examining the diet of predators that feed on diverse prey that vary substantially in exoskeleton composition (e.g., caterpillars versus adult beetle).

Besides prey remains, the other major source of nutrient deposition following feeding is excreta. Our results show that N deposition in excreta is not related to the N or protein content of the spider's meal. Our results are in contrast to previous studies that have shown that dietary nutrient content of food affects the nutrient content of consumer excreta (Sterner 1990, Elser and Urabe 1999, Sterner and Elser 2002). Relationships between nutrient intake and excreta

composition are more likely to occur when animals are well-fed and consuming more nutrients than can be utilized in the body. Yet, our experiments were conducted on spiders during the breeding season and under relatively food-limited conditions, as appears to be common for wolf spiders and other spiders in nature (Nyffeler and Benz 1988, Wise 1993, Wilder and Rypstra 2008). A long evolutionary history of food limitation may have selected for spiders to store as many nutrients as possible from each prey, even if it is biased in nutrient content, and to excrete as few nutrients as possible. In addition, producing eggs would provide females with a tissue source into which nutrients can be invested, which may also explain the high assimilation efficiency and low excreta production by spiders in our study. Similarly high assimilation efficiency and low excreta production might also be predicted for juveniles that are actively growing and building tissue. Further work is needed to test if the relationship between diet and excreta composition is dependent on developmental stage (juvenile versus adult), sex, or feeding level of the consumer.

Our results also show high variation in excreta nitrogen content. We believe this is a function of variation among individual spiders in excretion and not measurement error because: 1) measures of nitrogen in whole prey and prey remains have low variation, which suggests we can measure nitrogen precisely, 2) other measures of spider excretion (i.e., number of excreta produced and area of the substrate covered by excreta) are also highly variable, and 3) subsequent experiments measuring excreta of this species using different methods have found similarly high variation in excreta nitrogen content (Barnes and Wilder, Unpublished data). Further work is needed to determine why these spiders vary in excreta production. Regardless, excreta comprise a very small component of the overall nitrogen budget of the spiders (i.e., 1.6-2.3 % of total prey N, Figure 1) and, hence, even there had been statistically significant differences, they would likely have little ecological significance.

While the amount of nutrients deposited from individual prey may be small, there still may be situations where spiders or other predators have a significant effect on nutrient cycling. For example, even small deposited amounts of nutrients could have disproportionately large effects on ecosystem processes if they affect the structure and function of microbial communities (Hawlena et al. 2012). For example, in a grassland ecosystem, relatively small changes in the nutrient content of grasshopper carcasses had large effects on carbon mineralization, presumably through their effects on microbial communities (Hawlena et al. 2012). Second, while individual deposits of nutrients may be small, sedentary predators may deposit nutrients in a relatively small area over an extended period of time and, hence, have a significant impact on local nutrient deposition relative to patches without a predator. Finally, spiders may deposit larger amounts of nutrients when they feed on prey larger than can be fully consumed or prey that are over abundant (Samu and Bíró 1993, Riechert and Maupin 1998). Evidence also suggests that some species can preferentially extract particular nutrients from prey to meet their diet requirements, which would result in deposition of carcasses with biased concentrations of unconsumed nutrients (Mayntz et al. 2005). Further research is needed to examine how these and other conditions affect the amount of nutrients deposited by spiders and how these deposited nutrients affect ecosystem processes.

Predators are expected to accelerate nutrient cycling by consuming prey and converting the nutrients in prey tissue to nutrients that are immediately deposited in the environment in forms that may be rapidly incorporated by microbes and plants (Romero et al. 2006, Schmitz et al. 2010). It is important to understand how this effect of predators may be altered by changes in the nutrient content of their food, as prey nutrient content can vary widely within and among species both spatially and temporally (Fagan et al. 2002, Raubenheimer et al. 2007, Lease and Wolf 2010, Wilder et al. 2013). Whether or not predator excretion is affected by variation in the nutrient content of prey has important consequences for understanding how variation in trophic interactions will affect the flow of nutrients through ecosystems. Studies of aquatic systems have

shown that mass balance between food and consumer can be used to predict the nutrient content of excreta (Sterner 1990, Elser and Urabe 1999, Sterner and Elser 2002). Yet, the results of the current project demonstrate that spiders only release a small fraction of prey nutrients as excreta and the nutrient content of this excreta is not affected by the nutrient content of prey. Hence, spatial and temporal variation in the nutrient content of prey will have little if any effect on the ratio of nutrients deposited by this spider. Further work is needed to resolve the consequences of prey nutrient content for predator ingestion and excretion among a wider range of taxa, and whether the pattern observed in the current study represents a special case for spiders or more general differences between aquatic and terrestrial systems.

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Table 1 Composition of artificial diet treatments fed to crickets: 100:0:0, 80:10:10, 60:20:20, and 10:45:45 (protein:carbohydrate:lipid percentages) (in grams).

	10:45:45	**60:20:20**	**80:10:10**	**100:0:0**
Egg white	11	89	120	137
Micellar Casein	11	89	120	137
Sugar	55	20	7	0
Flour	85	35	14	0
Cellulose	94	51	33	26
Nipagin	1	1	1	1
Vitamin (Capsule)	1	1	1	1
Cholesterol	0.5	0.5	0.5	0.5
Fish Oil	3	3	3	3
Lard	22	8	3	0
Olive Oil	22	8	3	0

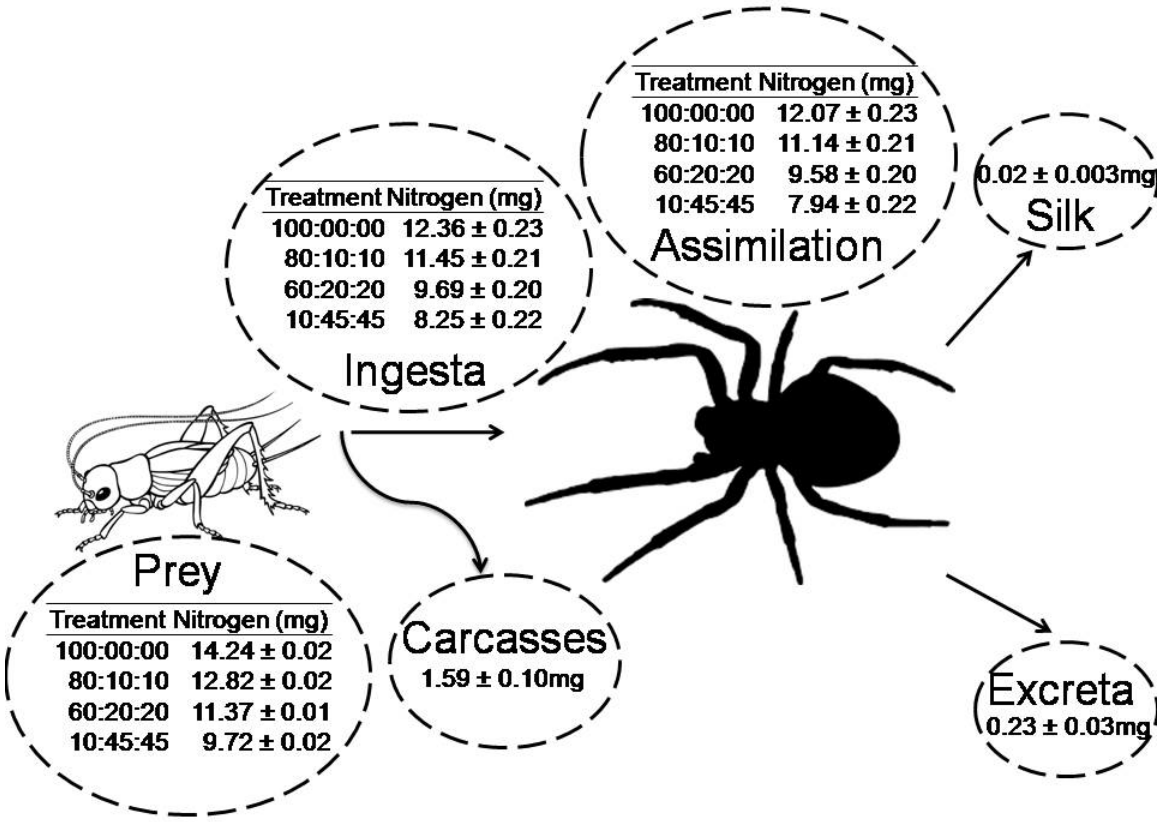

Figure 1. Diagram showing the potential fates of prey nutrients. Nitrogen in prey bodies can be either ingested or deposited as uneaten remains following feeding. Those nutrients that are ingested can then be either assimilated, excreted or deposited as silk. Numbers represent a summary of data from the experiment. Circles with multiple numbers are those compartments where there were significant differences in nutrients between the cricket diet treatments. Circles with only one number are those where there was no significant difference in nutrients between the cricket diet treatments. There was significant variation in the nitrogen content of prey, nutrients ingested by spiders and nutrients assimilated by spiders but there was no significant difference among treatments in prey remains, excreta or silk. Most prey resources were assimilated by spiders rather than deposited.

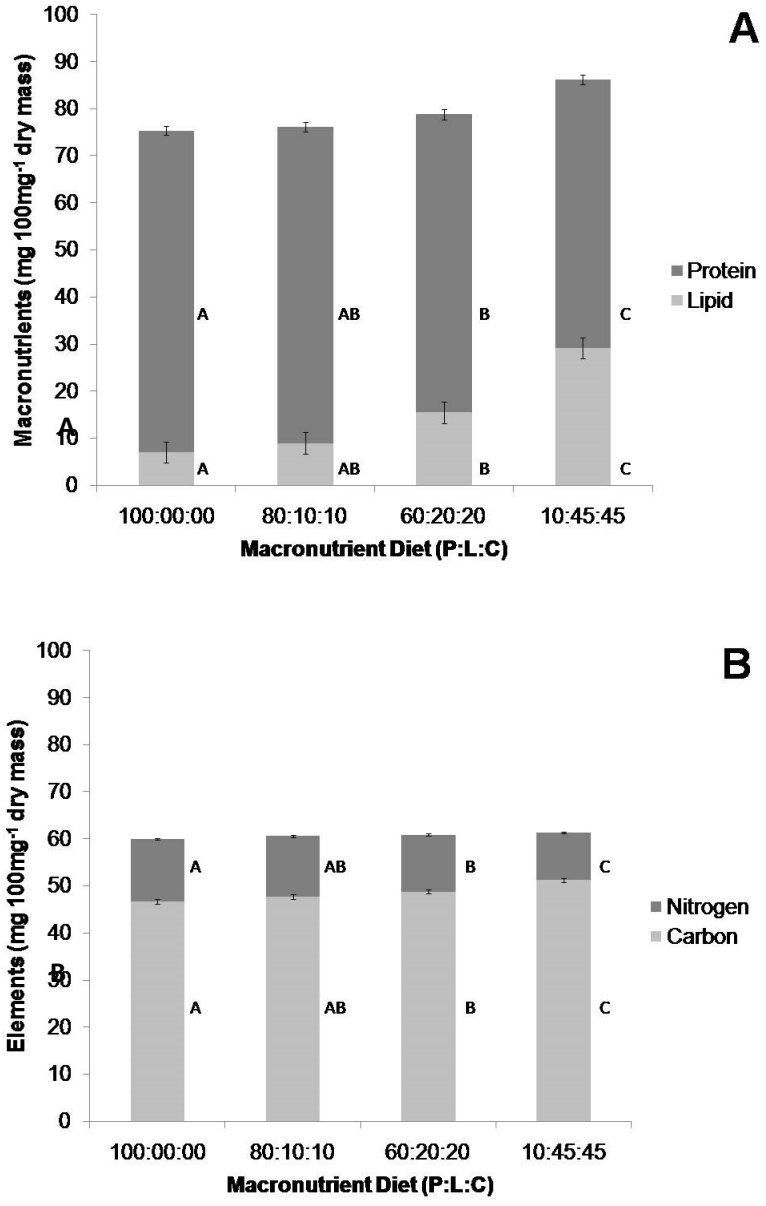

Figure 2. Macronutrient (A) and elemental (B) composition of crickets on artificial diet treatments (protein:carbohydrate:lipid percentages) (Mean + 1 SE). Bars with different letters were significantly different from each other in post hoc analyses.

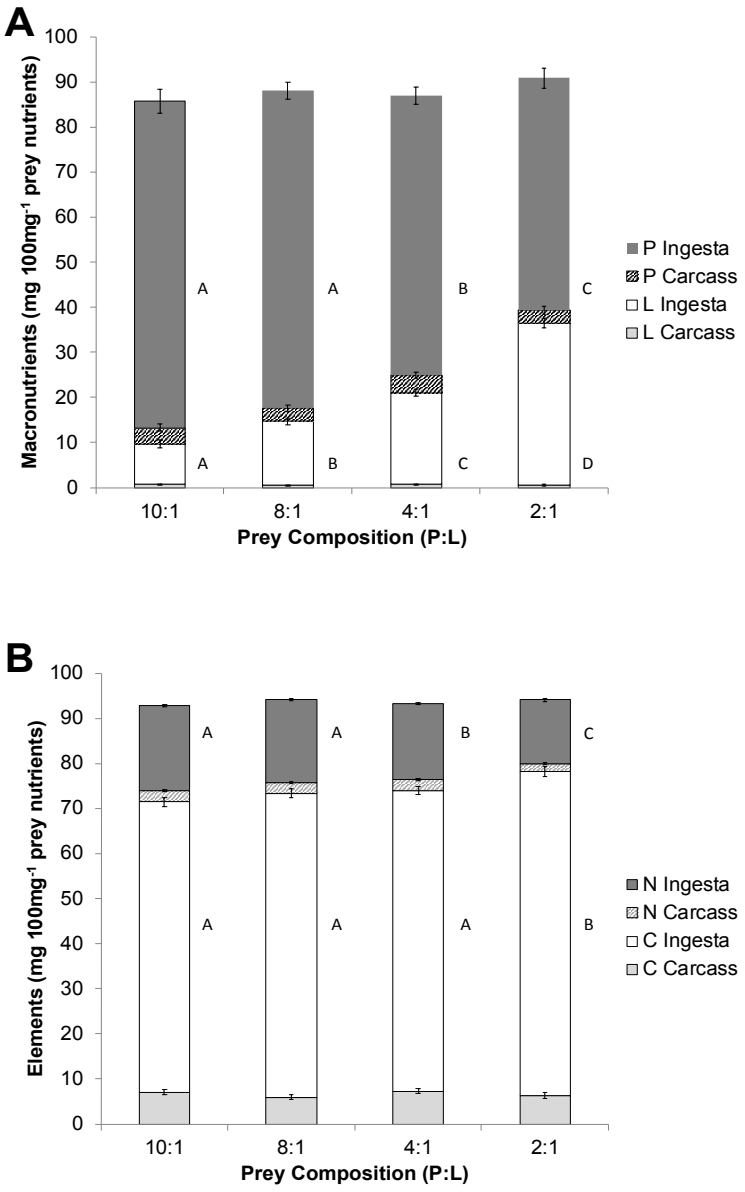

Figure 3. Prey macronutrients (A) and elements (B), relative to the total prey nutrients, in the wolf spider's cricket prey remains and ingesta by prey composition (protein:lipid from Figure 2A). (Mean + 1 SE). Bars with different letters were significantly different from each other in post hoc analyses.

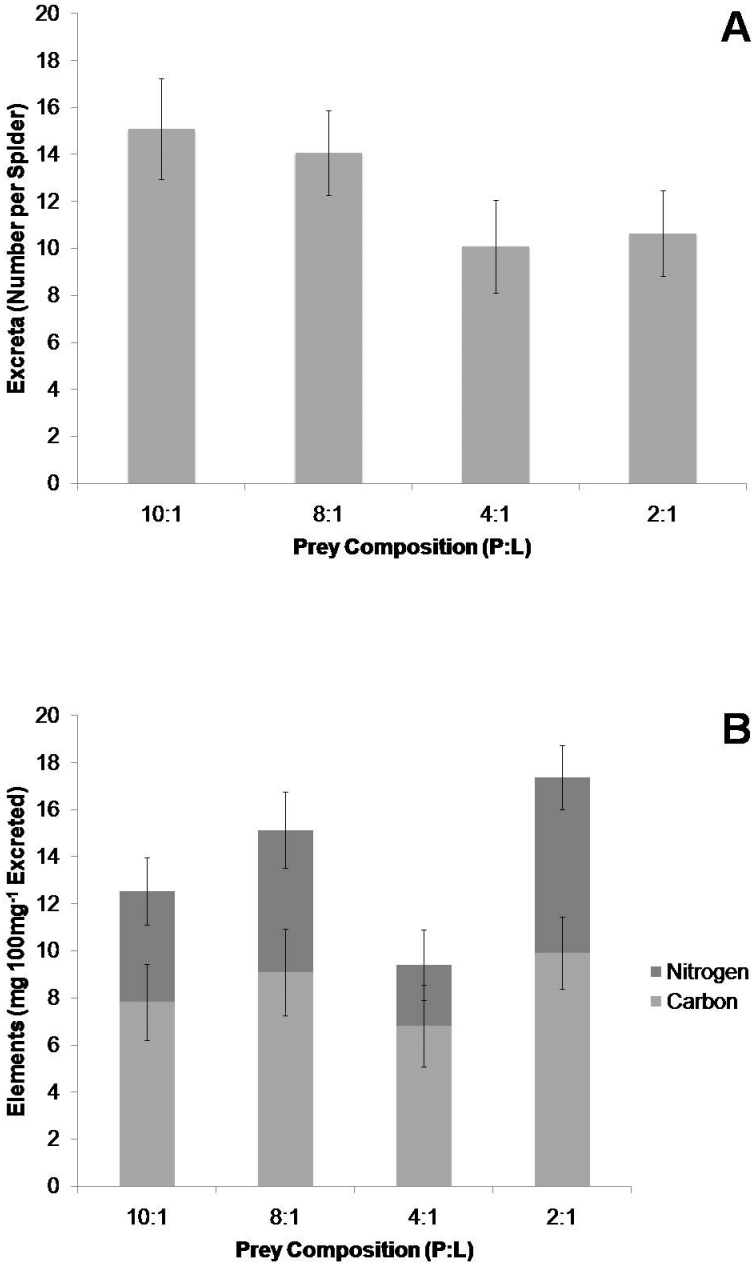

Figure 4. Quantity (A) and elemental composition (B) of excreta produced by wolf spiders fed prey of different composition (protein:lipid from Figure 2A) (Mean + 1 SE).

CHAPTER II

Consequences of Prey Exoskeleton Content for Predator Feeding and Digestion

hunting predator

Abstract

Predators often feed on a wide range of prey that can vary in behavior, morphology, and physiology. The net benefits that predators gain from prey are likely related to both prey nutrient content and prey morphology or defenses. For invertebrates, the exoskeleton is a morphological trait that varies widely among species and could affect nutrient extraction by predators. The goal of this study was to determine how prey exoskeleton content affected predator nutrient intake, assimilation, and excretion by comparing spiders feeding on either larval or adult mealworms of similar size. We found that the proportion of prey energy invested in digestion was greatest in spiders consuming adult mealworm beetles which had higher amounts of exoskeleton than larvae. Further, spiders extracted a greater proportion of elements, macronutrients, and energy from the larval mealworms, which had lower amounts of exoskeleton. Interestingly, total nitrogen content of prey was not a predictor of nitrogen assimilation as spiders assimilated more nitrogen from the larval mealworms, which had lower total nitrogen content. While adult beetles had higher total nitrogen content, their discarded remains of prey had large amounts of nitrogen that was nutritionally unavailable for spiders (i.e., exoskeleton). These results suggest that prey exoskeleton can affect assimilation efficiency by predators, and that a combination of macronutrient and elemental analyses may be needed to examine the quality of prey for predators

and the potential consequences of predation for nutrient flows (e.g., consumer assimilation, egestion, and excretion) in ecosystems.

Introduction

Many predators are polyphagous and their potential prey can vary in a number of traits including: nutrient content, behavior (e.g., evasion), morphology, crypsis, and toxicity (Denno and Fagan 2003, Fagan and Denno 2004). These traits could impact predator-prey interactions in a number of ways. First, prey traits can affect the ability of predators to locate or subdue prey. Second, prey traits can affect prey choice by affecting the attractiveness of prey to predators (e.g., toxicity). Third, once a prey is captured, prey traits can affect handling time and the ability of predators to efficiently extract nutrients from prey. One factor that could influence prey capture and handling is prey exoskeleton content. Prey can vary substantially in exoskeleton content (9 – 60% of dry mass) from soft prey (e.g., larval insects such as caterpillars) to heavily sclerotized prey (e.g., beetles; Kaspari and Joern 1993, Lease and Wolf 2010). Prey exoskeleton has been suggested to be a factor that affects the ability of predators to subdue prey and affect the choice of prey by predators. Yet, less is known about the role of prey exoskeleton content for mediating predator digestion and nutrient extraction.

Many vertebrate and invertebrate predators feed on arthropods. In particular, spiders are among the most abundant and diverse carnivores within terrestrial ecosystems. Spiders also consume a significant amount of prey worldwide, which has been estimated to be 400-800 million tons per year (Nyffeler and Birkhofer 2017). Spiders, like most arthropod predators, feed using extra-oral digestion (Cohen 1995). This feeding mode clearly separates undigested parts of prey (e.g., egesta) and excreta and provides a model system for studying how indigestible components (i.e., exoskeleton) of prey affect the ingestion of digestible nutrients. Digestible nutrients (e.g.,

lipid and protein) are liquefied; filtered through the mouth and pharynx; digested; and either assimilated, respired or excreted as nitrogenous by-products (Foelix 2011). Predators that feed using extraoral digestion can be very efficient at liquefying nutrients in the soft tissues of prey, leaving behind uneaten prey remains, which are largely composed of indigestible exoskeleton. The inability of spiders to digest exoskeleton, like many other predators of invertebrates, means that the energy and protein (e.g., cross-linked proteins bound in the chitinous matrix, which can be up to half the weight of exoskeleton) in this compound are not nutritionally available to spiders (Klowden 2013). While previous research has examined the use of digestible nutrients in prey, less well-known are the consequences of prey exoskeleton content for the energetic costs of prey handling, digestion, and nutrient extraction by spiders.

The energetic cost of handling, digesting, and assimilating nutrients has been termed Specific Dynamic Action (SDA). The SDA coefficient refers to the proportion of the total ingested energy that is spent as SDA. Two open areas of research in this area are the physiological factors contributing to variation in SDA and the characterization of SDA in novel organisms (McCue 2006). Although SDA has been investigated in numerous taxa (Jobling 1983, McCue 2006, Secor 2009), studies of SDA in spiders are particularly sparse. Nespolo et al. (2011) was one of the first to measure SDA in spiders and found that spiders do elevate respiration following digestion (i.e., they do have a SDA response). Jensen et al. (2010) studied SDA in wolf spiders and found no effect of prey macronutrient content on spider respiration. Links between metabolism, nutrient allocation, and nutrient flows will likely produce key insights into predator feeding and digestion and its consequences for ecosystems.

The overall goal of this study was to determine how prey exoskeleton content affected nutrient extraction and the metabolic costs of digestion (i.e., SDA) in a predator. We did so by feeding similarly sized larval or adult beetles to black widow spiders (*Latrodectus mactans*), which are generalist, web-building spiders. By using these prey treatments, we were able to test the effects of exoskeleton content (i.e., low in larvae and high in adults) on feeding and digestion while controlling for prey size, species identity, and prey diet. We measured the ingestion, egestion (uneaten remains of prey), assimilation, and excretion of black widow spiders feeding on larval and adult mealworm beetles (Figure 1). Further, we measured the metabolic costs of processing a meal (i.e. SDA) using closed-system respirometry. We predicted that spiders consuming prey with a greater amount of exoskeleton (i.e., adult beetles) would extract fewer resources from their prey, leaving a greater proportion of nutrients in the prey remains. We also predicted that SDA would increase with exoskeleton content due to the potentially higher costs of puncturing and sucking nutrients from prey with rigid exoskeletons.

Methods

Study Species: *Tenbrio molitor* larvae were purchased from a commercial distributor (Fluker Farms, Louisiana) and used to create a breeding population that produced a constant supply of larvae and adults. The colony of mealworm larvae and beetles were maintained on a diet of wheat germ and provided potatoes as a water source. The colony was maintained at constant 25 ± 1 OC and 14L:10D light regime.

Female black widows (*Latrodectus mactans*) were collected from residences in Stillwater, Oklahoma during summer 2016. These spiders produced egg sacs in the lab and the spiderlings were reared to maturity (n= 30). The spiders were maintained at a constant 25 ± 1 oC and 14L:10D light regime in the lab. They were lightly misted with water and fed twice per week on vinegar flies (*Drosophila melanogaster*) and crickets (*Acheta domesticus*) of roughly half of each spider's body mass. Once mature, the spiders were housed in 946 mL (32oz), clear plastic deli containers. In the field, black widow spiders often feed on Coleopterans (Salomon 2011). All applicable institutional and/or national guidelines for the care and use of animals were followed.

Feeding Trials: Our study used a standardized starvation period to clear the spider gut of previous meals. Spiders were fasted for 14 days prior to each feeding trial. In the field, spiders often experience starvation periods greater than one week (Bilde and Toft 1998). For example, in another species of spider, body condition of individuals collected from the field was not significantly different from laboratory individuals that were completely deprived of food for 3 months (Wilder and Rypstra 2008). In addition, a starvation period is critical for motivating spiders to feed and ensuring that the results of the study measure

maximum extraction ability when feeding on the prey treatments. On day 9 of the fast, we measured spider body masses (± 1mg) and transferred them to individual metabolic chambers to quantify baseline metabolic rate prior to feeding trials (see below). Spiders were then ordered by decreasing mass; even numbers were assigned to mealworm larvae and odd numbers were assigned to adult beetle treatment groups. This assignment, rather than one designated at random, ensured similarity of spider mass between prey treatment groups. Five control chambers were established the same as the experimental groups, minus the organisms.

Following the14-day fast, spiders were fed a single pre-weighed prey item (i.e. larval or adult beetle). The wet mass of each larva (80.9 ± 3.2mg) and beetle (78.0 ± 3.1mg) were selected from the breeder colony such that there was no difference in mass between the prey treatments ($t_{1,27}$= 0.65, p= 0.52). Prey mass in proportion to the spider mass (i.e. relative prey mass) did not differ, either. More specifically, there were no differences between relative prey mass of larva (25.8 ± 1.9%) and beetles (24.0± 1.8%; $t_{1,27}$ = 70, p= 0.49). The mass of late-development larval insects such as mealworms is often similar to or larger than that of recently eclosed adults as metamorphosis involves the metabolism of a significant amount of tissue for energy.

After prey were introduced, spiders were observed every three hours and the time at which prey were discarded was recorded. The discarded prey remains were removed and weighed after 9 hours to ensure that all spiders had standardized time available to feed on prey. All spiders had finished feeding by 9 hours. Excreta production was monitored daily for the next 5 days. When excreta were found in the spider containers, the excreta were collected (see "Nutritional Analyses" below) and the container was

exchanged for a clean one. Spiders generally captured prey within 30 seconds, completed extra-oral digestion in 6 hours, and produced most of their excreta within 24 hours. One black widow failed to feed and was excluded from subsequent analyses.

Metabolic Rate: We measured spider metabolism using closed-system respirometry to quantify accumulated CO_2. Respiration, feeding, and excreta production of spiders were monitored within tall 946 mL deli containers with lids modified to include sealable ports. The lower half of each container was lightly abraded with sandpaper to permit climbing and web production, but the upper half was left smooth to minimize climbing and interference with the container ports.

Standard metabolic rate (SMR) of post-absorptive spiders was measured every 12 hours (09:00 & 21:00) for the final two consecutive days of the fast. After the spiders were presented with prey, the chambers were re-sealed and respiration measurements were collected at an interval of three hours for the duration of the next 12 hours. All prey were removed at 9 hours to prevent decomposition of prey from affecting respiration measurements. Following this sampling duration, measurements were resumed at the 12-hour interval for the next 5 days. The five-day post-feed sampling duration exceeded the time required for metabolic rates to return to baseline.

At the end of each respirometry period, we flushed metabolic chambers using a small aquarium aerator pump after which we recorded CO_2 levels within each chamber. The experimental groups' chambers were rotated within the shelving unit at the end of each sampling period and locations of individual chambers were randomly assigned to intersperse potential spatial variability within the housing space. Respiration was

measured in accumulated CO2 (ppm) using a Li-840A CO2/H2O gas analyzer (LI-COR Biosciences) and converted into percent by dividing by 10000. To calculate VCO2 this was then divided by 100 and multiplied by the volume of the container (946 mL). The metabolic rate was calculated as the end minus start (i.e., just after chambers were flushed with the aquarium pump) respiration readings, divided by the sampling duration. Metabolic rate was converted to energy using the conversion factor 24.65kJ L CO_2^{-1} (Chown et al. 2007)

We characterized multiple components of the post-feeding metabolic profile. The peak VCO_2 was defined as the highest metabolic rate (CO_2 hr^{-1}) following feeding. The scope was calculated as peak VCO_2 divided by the baseline SMR. CO_2 production during each time interval was converted to units of energy and summed for calculation of SDA. The end of SDA response was conservatively identified as the time-step at which post-feeding respiration was no longer statistically different from the SMR. The SDA coefficient was determined by dividing the ingested prey energy by SDA.

Nutritional Analyses: We froze the whole prey, prey remains and excreta until analyses. Whole prey and remains were then dried at 60 ^oC for 24 hours, bisected, and weighed. Then, each half was randomly assigned to either macronutrient or elemental analysis. Excreta was suspended in 0.1M sodium hydroxide prior to elemental analysis.

We determined lipid content as change in mass following sequential soaking and extraction in chloroform over the course of three days (Wilder et al. 2013). Protein content was determined in triplicates using the Bradford Assay on lean, ground samples (Wilder et al. 2013). Protein analysis using the Bradford Assay on invertebrates only

measured the protein present in the soft tissues of the prey (Wilder et al. 2013). Exoskeleton can have considerable protein content; however, proteins present within the exoskeleton are unavailable to consumers because they are bound within the inedible matrix of chitin. Hence, sclerotized proteins in the exoskeleton are not included in our measures of protein. Carbohydrates were not measured as they are typically present at low levels in arthropods (Raubenheimer and Rothman 2013). Nutrient dry masses were converted to energy using standard conversion factors (protein = 17 kJ/g, and lipid = 37 kJ/g; Raubenheimer and Rothman 2013).

Lipid extracted whole-prey, lipid from prey, uneaten remains of prey, and spider excreta were evaluated for carbon and nitrogen content. We removed lipid from prey items before elemental analysis because high lipid content of prey, especially mealworm larvae, can make it difficult to homogenize samples. Hence, to calculate total carbon content of whole prey we also measured the carbon content of the purified lipids that were extracted from prey and, based on the lipid content of prey, factored this back in to the calculation of prey total carbon content. The C and N in samples were quantified from combustion in an elemental analyzer (Elementar Americas, Inc., Mt. Laurel, New Jersey). We used wet mass and nutrient content of control larvae and beetles to develop linear regression equations. From the linear equations, we were able to use the wet mass of prey fed to spiders to estimate the masses of each nutrient contained in the prey before it was fed to a spider. Ingesta was calculated as the elements and macronutrients estimated in prey before they were fed on by spiders minus the contents of the prey remains. Elemental assimilation was calculated as the carbon and nitrogen estimated to be in prey before they were fed on by spiders minus the carbon and nitrogen in the prey remains and

excreta. Energy assimilation was calculated as the difference in initial prey energy minus energy in prey remains and SDA.

We determined exoskeleton mass from lean (i.e., lipid extracted) whole mealworm larvae and adult beetles. Tenebrio were soaked in 8mL of 0.1M sodium hydroxide, and sonicated in a hot water bath at 80 ºC for 2 hours. Sodium hydroxide dissolved the soft tissue in the body (Lease and Wolf 2010). Finally, mealworm larvae and adult beetle exoskeletons were removed, dried at 60 ºC, and re-weighed to quantify exoskeleton mass. Exoskeleton content was quantified as exoskeleton mass divided by the dry mass of the prey.

Statistics: Differences in prey mass, nutrient content, and energy were examined using t-tests and ANOVA in JMP 12 software package (SAS Institute, Cary, NC, USA). The SDA was compared between prey types using repeated measures mixed models MANCOVA in the SAS statistics program, with spider mass as a covariate. The Least Squares Means of the SMR and post-feeding metabolic rates were plotted from the mixed models MANCOVA. Termination of the SDA was the time-step at which respiration no longer significantly differed from the baseline, determined from differences of Least Squares Means post hoc tests.

Results

Prey Content: The chemical composition of mealworm larvae and adult beetles differed considerably. Larvae had over 3 times higher lipid and slightly higher protein than adult beetles. Consequently, mealworms had significantly higher energy content compared to adult beetles (Table 1). Larval mealworms also contained slightly higher carbon and slightly lower nitrogen content than adult beetles (Table 1). Adult beetles had almost twice as much exoskeleton content compared to mealworm larvae (Table 1).

Macronutrient and Elemental Digestion: Following feeding, the prey remains of adult beetles weighed significantly more than the prey remains of larvae ($t_{1,27}$= -12.44, p< 0.0001). Spiders ingested greater lipid ($t_{1,22}$= 17.11, p< 0.0001) and protein ($t_{1,22}$= 3.24, p= 0.0038) from mealworm larvae compared to adult beetles (Figure 2). Spiders left behind a greater amount of protein in prey remains following feeding on adult beetles ($t_{1,22}$= -3.74, p= 0.01) compared to larvae, but there was no difference in lipid in prey remains between prey types ($t_{1,22}$= 1.04, p= 0.31; Figure 2). The uneaten remains of adult beetles also contained greater carbon ($t_{1,27}$= -5.26, p < 0.0001; Figure 3A) and nitrogen ($t_{1,27}$= -5.22, p < 0.0001; Figure 3B) compared to those of mealworm larvae. Excreta from black widows fed mealworm larvae and adult beetles did not differ in dry mass of carbon ($t_{1,27}$= -0.32, p= 0.75) or nitrogen ($t_{1,27}$= -0.08, p= 0.94; Figure 3A and B). Overall black widow assimilation was greater when feeding on mealworm larvae, compared to adult beetles, in both carbon ($t_{1,27}$= 6.08, p< 0.0001) and nitrogen ($t_{1,27}$= 2.40, p= 0.02; Figure 3A and B).

Digestive Energetics: Spider mass ($F_{1,25}$= 3.98, p= 0.06) and time ($F_{11,275}$= 5.93, p < 0.0001), but not treatment ($F_{1,25}$= 1.04, p= 0.32), influenced black widow post-feeding metabolism (Figure 4). The peak metabolism occurred at 9 hours post-feeding and was greater in spiders digesting larvae than adult beetles ($t_{1, 275}$= 4.25, p < 0.0001). Widows fed adult beetles both expended greater proportions of the digestible prey energy on SDA ($t_{1,27}$= -3.90, p= 0.0006; Figure 5) and deposited more energy in prey remains ($t_{1,22}$= -2.17, p= 0.04). Consequently, widows assimilated relatively less energy ($t_{1,22}$= 2.96, p= 0.0073; Figure 5) and had significantly higher SDA coefficients (Table 3) when feeding on adult beetles compared to larvae.

Discussion

These results demonstrate that the exoskeleton content of prey affects the extraction and assimilation of nutrients as well as the digestive energetics of arthropod predators. Specifically, the high exoskeleton content of adult beetles resulted in reduced macronutrient, element, and energy ingestion relative to larval mealworms by black widows. However, spider SDA, handling time, and passage rate did not differ between prey types. The total amount of excreta produced by spiders was also very low and did not differ between prey types. Differences in the composition of prey appear to change the relative amounts of nutrients that are assimilated versus egested – with less assimilation and greater egestion of nutrients from prey containing higher exoskeleton content (Figure 1). Understanding the consequences of prey exoskeleton content for predator digestion and assimilation is important as arthropod prey vary widely in exoskeleton content and many predators feed on a diversity of prey (Kaspari and Joern 1993, Fagan and Denno 2004, Lease and Wolf 2010).

Our results suggest that total nitrogen content of prey may not be a reliable indicator of the quality of prey for predators. From a whole-prey perspective, adult beetles had higher total nitrogen content than larval mealworms (Table 1). Yet, spiders discarded more nitrogen and ingested less nitrogen when feeding on these adult mealworm beetles relative to larvae. The low assimilation efficiency of nitrogen when feeding on adult beetles is likely because they contained large amounts of exoskeleton. Exoskeleton can have a significant amount of protein bound within the inedible chitin matrix and this protein is inaccessible to most predators (Klowden 2007).

Indeed, analysis of macronutrients (i.e. lipid and proteins) in whole prey items provided a better predictor of what would be assimilated versus discarded following feeding by predators. Spiders consumed nearly all lipid and protein in prey and the discarded remains were nearly completely composed of indigestible exoskeleton. Prior attempts have been made to use total nitrogen content of prey as a measure of their nutrient content or quality for predators (Fagan et al. 2003, Denno and Fagan 2003, Fagan and Denno 2004). Yet, the conclusions of these studies were contradicted by parallel studies that measured the macronutrient content of prey and digestion by predators (Wilder and Eubanks 2010, Wilder et al. 2013). The current results comparing assimilation based on nitrogen and protein measures explain why previous studies provided different conclusions (i.e., prey vary in exoskeleton content, which can be a significant pool of indigestible nutrients including N). Furthermore, this suggests that further work is needed to better reconcile or combine elemental and macronutrient currencies for measuring nutrients.

Elements are an important currency because they can be followed from individuals to ecosystems throughout the cycle. Yet, elements may not be as useful for predicting variation in feeding preferences and nutrient use efficiencies as macromolecules when food items vary significantly in the amounts of elements (e.g., N) present in digestible (e.g., protein) and indigestible (e.g., exoskeleton) molecules. A way to have the best of both worlds might be to use biochemical measures (e.g., protein and exoskeleton) to quantify different pools of nitrogen (e.g., digestible N versus indigestible N) (Leroux et al. 2012).

The digestive metabolic response of many consumers to various prey-types has been investigated (Jobling 1983, McCue 2006, and Secor 2009). In previous studies of prey types, "hard-bodied" prey (e.g. Zophobas beetle larvae with chitinous exoskeletons) were generally more energetically costly to digest than "soft-bodied" prey (e.g. Lumbricus earthworms) by amphibians (Secor and Faulkner 2002, Secor and Boehm 2009) and reptiles (Britt and Bennett 2008). That is, consumers experienced greater SDA, peak metabolism, and duration of SDA when digesting more chitinous prey in these studies. Broadly, greater energetic costs of digestion could also reduce the allocation of limited energy to activity and other budget components (Boggs 2009). Such costs and their consequences on life history should be considered when examining the quality of different prey for predators.

Organisms incapable of chewing, such as black widows, may require greater effort to puncture or suck contents from within the relatively rigid arthropod prey exoskeleton that do not readily collapse as nutrients are removed. Black widows would therefore be predicted to have increased handling costs reflected by SDA. However, only peak digestive respiration and SDA coefficients differed between prey treatments. The spiders did not differ in handling time between mealworm larvae or adult beetles. The end of prey handling time generally corresponded with the times at peak respiration (Figure 4), suggesting that the greatest energetic cost contributing to SDA primarily occurred during extra-oral digestion. It is not yet clear if this cost is due to the capturing, puncturing, sucking, or another process. More detailed observations of these behaviors with finer temporal resolution could elucidate the leading mechanism. Smaller sampling intervals, or the use of flow-through respirometry, would also be necessary to identify the

timing of SDA peak. Nevertheless, we were able to identify a difference in macronutrient ingestion and element assimilation (Figures 1, 2, and 3A-B). Differences in the total amount of digestible energy in prey (i.e., larvae have higher total energy content than adult beetles) contributed to a higher SDA coefficient. Hence, our findings suggest that the primary consequence of increases in prey exoskeleton content is reduced nutrient intake and not necessarily a large increase in digestive metabolism.

Nutrient assimilation efficiency is particularly high in spiders. Most of the available lipid and protein in prey were ingested by spiders, rather than deposited in prey remains (Figure 2). After deducting prey remains and SDA energy from the original prey content, we found that spider energetic assimilation efficiency ranged between 84-91% (Figure 5). Although relatively high in both treatments, spiders consuming prey with less exoskeleton (i.e. larval mealworms) had higher energetic assimilation efficiency. Spiders deposited slightly but significantly greater energy, protein, and exoskeleton from adult beetles, compared to mealworm larvae, as uneaten remains in the prey (Figure 5). The rigid exoskeleton of adult beetles likely prevented spiders from efficiently extracting all the nutrients in these prey.

The differences in resource intake and assimilation efficiency suggest that prey exoskeleton content has significant consequences for predator feeding and digestion and that measures of macronutrients, and not elements, may provide a better understanding of the consequences of feeding on different prey, especially when prey vary in exoskeleton content. The costs (e.g., reduced assimilation efficiencies for energy and nutrients) of feeding on prey with higher exoskeleton could influence the foraging decisions and life history of predators. Behavioral studies will be useful to examine this possibility. In

addition, when predators do feed on prey, the exoskeleton content of those prey may affect nutrient deposition by predators. Spiders assimilated less and deposited more nitrogen, as both protein and exoskeleton, in prey remains when feeding on prey with higher exoskeleton content. Although exoskeleton is slow to decompose, these nutrients likely still, eventually, provide a significant contribution to nutrient cycling in ecosystems – especially given worldwide estimates of prey consumption by spiders (Seastedt and Tate 1981, Nyffeler and Birkhofer 2017). Hence, in addition to nutrient content of prey, our results suggest that future studies of prey quality and foraging by spiders and other polyphagous predators should also measure and consider the role of prey exoskeleton in the costs and consequences of feeding on different prey.

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58.

Table 1 Nutritional composition (mean ± SE) of *Tenebrio molitor* larvae and adult beetles, expressed relative to dry mass. Total nitrogen includes content within the exoskeleton.

Component	Larvae	Beetles	$t_{1,42}$	P
Lipid (mg)	10.1±0.17	2.9±0.18	29.13	**< 0.0001**
Protein (mg)	23.8±0.43	21.0±0.45	4.49	**< 0.0001**
Exoskeleton (mg)	3.8±0.14	7.2±0.15	-16.64	**< 0.0001**
Carbon (%)	51.5±0.05	49.0±0.05	37.50	**< 0.0001**
Nitrogen (%)	8.3±0.06	10.7±0.05	-31.46	**< 0.0001**
Energy (kJ g^{-1})	22.5±0.10	15.5±0.10	67.00	**< 0.0001**

Note: Significant differences between prey types (t-test) are shown in bold.

Table 2 Metabolic variables (mean ± SE) of black widows fed *Tenebrio molitor* larvae and adult beetles.

Component	Larvae	Beetles	t	P
N	14	15		
Spider Body mass (g)	0.3340±0.02	0.3430±0.02	-0.27	0.78
Prey Size (g)	0.0809±0.003	0.0780±0.003	0.65	0.52
Prey Size (% body mass)	25.8±1.9	24.0±1.8	0.69	0.50
SMR VCO_2 (mL hr^{-1})	0.0139±0.007	0.0156±0.007	-0.16	0.87
Peak VCO_2 (mL hr^{-1})	0.2084±0.007	0.1666±0.007	4.25	**<.0001**
SDA Duration (hrs)	24	24		
Scope	15	10.7		
SDA (kJ)	0.04±0.002	0.03±0.002	1.74	0.09
SDA Coefficient (%)	4.93±0.50	7.74±0.48	-4.04	**0.0004**
Handling Time (hrs)	8.36±0.4	8.20±0.3	0.32	0.75
Passage Rate (hrs)	24.0±0	24.0±0	-	-

Note: Significant differences between prey types (t-test) are shown in bold.

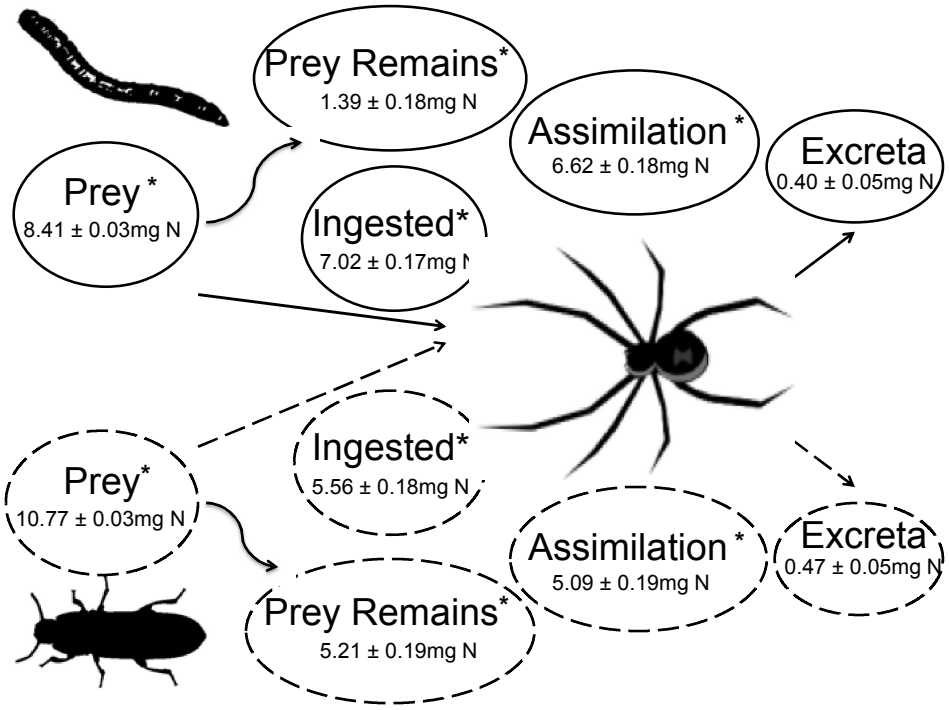

Figure 1. Diagram showing the fates of prey nitrogen, standardized for a 100mg dry mass prey. Nitrogen in prey can be either ingested or deposited as uneaten remains of prey following feeding. Those nutrients that are ingested can then be either assimilated or excreted. Solid circles and arrows mealworm larvae and dashed circles and arrows represent adult beetles. Values are expressed as means ± SE and asterisks indicate significant differences between prey treatments.

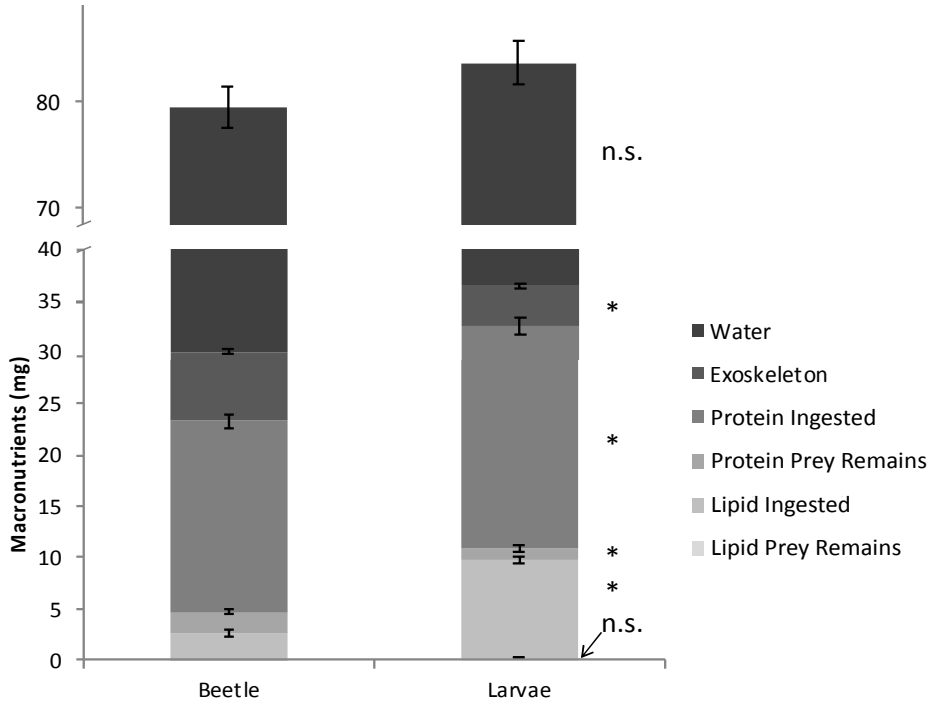

Figure 2. Partitioning of prey macronutrients (wet mass) by black widows into the spider's prey remains and ingesta by prey treatment. Asterisks indicate significant differences between prey treatments. Lipid content in prey remains was low and non-significant between prey types, indicated by an arrow.

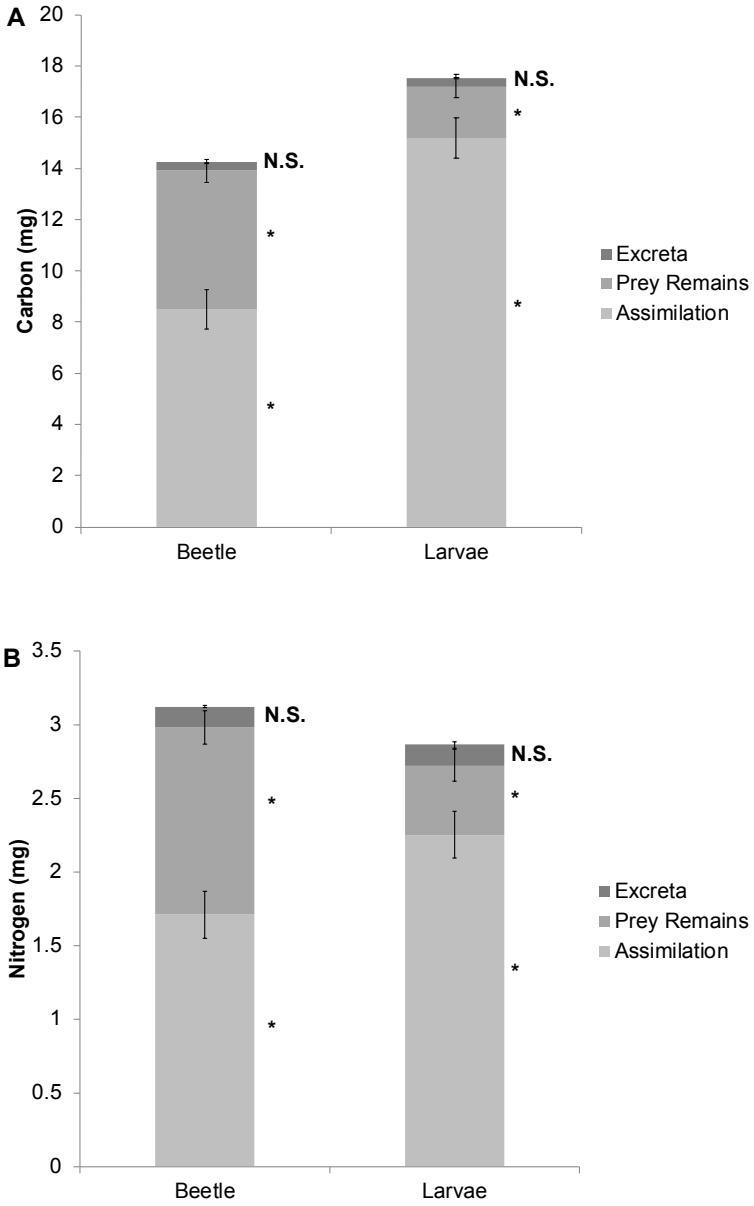

Figure 3. Allocation of elements within assimilation, prey remains, and excreta by black widows (A & B). Asterisks indicate significant differences between prey treatments.

CHAPTER III

Consequences of Prey Nutrient Content for Spider Growth and Nutrient Cycling

Abstract

Consumer-driven nutrient recycling has the potential to broadly drive patterns in ecosystem function. Ecological stoichiometric theory predicts that mass balance will drive excretion when consumers feed on prey that varies in nutrient content. Another prediction is that nutrients excreted are negatively related to the consumer's body nutrient composition. Further, nutrient deposition is a critical link between aboveground and belowground communities. Our goal for this study was to test how variation in prey nutrient content affected spiders and the contribution of spiders and prey to soil nutrient dynamics, including soil respiration and subsequent plant growth. We used either high-lipid or high-protein prey additions to microcosms for testing if the effects of predators on soil dynamics vary with soil fertilization level. We predicted that the presence of spiders would significantly increase soil respiration and plant growth, especially at the lowest levels of fertilization, but that diet would not impact these effects. We found that spiders grew most on the high-lipid diet, but that this difference due to diet did not result in distinct soil and plant dynamics. However, the nutrients deposited by spiders and prey carcasses did impact soil and plant dynamics at the lowest level of soil fertilization. Our findings suggest that spiders are important suppliers of labile nutrients to soils, but more

importantly that the processing of spider excrement by soil communities initiates decomposition of more complex organic nutrients and indirectly contributes to plant growth.

Introduction

The rates and ratios by which animals recycle nutrients are thought to be a consequence of mass balance between the consumer's body composition and the nutrient content of their food (Elser and Urabe 1999, Vanni et al. 2002, Hood et al. 2005). Nutrients in excess of consumer demands will be returned to the environment (Sterner and Elser 2002). This theory of consumer-driven nutrient cycling has been developed and confirmed largely through studies of pelagic and aquatic primary producers and herbivores (Elser and Urabe 1999, Sterner and Elser 2002). For example, phosphorus-rich armor-plated catfish recycle less phosphorus when feeding on algae than do less phosphorus rich fish in the same environment (Vanni et al. 2002). While mass balance appears to predict nutrient cycling in some systems, it has yet to be critically evaluated in other systems including terrestrial systems and carnivores, in general (Sitters et al. 2017).

Predators link aboveground and belowground nutrient cycling through both direct effects and indirect effects. Predators can indirectly alter soil mineralization by causing prey to increase activity and C:N, via increased prey N excretion and body protein breakdown (Hawlena et al. 2012). Direct effects of carnivores are widely variable. Predators can strongly influence soil processes and plants through trophic cascades, prey consumption and deposition, translocation of nutrients across habitats, and other modes (Schmitz et al. 2010). For example, the location of wolf kill-sites increases spatial heterogeneity of soil and plant nutrients, creating localized patches of high productivity (Bump et al. 2009). Another example is the facultative mutualism between tropical jumping spiders and bromeliad plants, where spiders contribute a high quantity of nitrogen as excrement and bromeliads provide structural habitat for spiders (Romero et

al. 2006). Spiders are a diverse group and are a particularly novel predator model system for testing how nutrition shapes physiology, foraging behavior, and broad ecological processes (Wilder 2011).

Yet, while some studies have documented direct effects of predator excretion on ecosystem processes, other studies have documented that many predators can be food-limited, which should encourage predators to be efficient with nutrient assimilation and less likely to release significant amounts of nutrients to the environment (Wise 1993). For example, studies of spider foraging have demonstrated that they extract nearly all of the macronutrients present in prey bodies and primarily deposit prey exoskeleton into the environment (Wilder 2011, Wilder et al. 2013, Barnes et al. In revision). Arthropod exoskeleton is likely a low quality resource for soil microbes and decomposers as it can take years to decompose (Seastedt and Tate 1981). Hence, whether or not predators return significant amounts of nutrients to the environment in their excreta/egesta, and whether or not this nutrient deposition varies with prey nutrient content, as predicted by consumer-driven nutrient cycling, remain unclear.

Spiders are diverse and abundant carnivores in many terrestrial systems. Annually, spiders consume an estimated 400-800 million tons of prey per year, which is an amount comparable to the approximated 400 million tons of meat consumed by humans annually (Nyffeler and Birkhofer 2017). Spiders are polyphagous and prey nutrient content can vary widely among species in nature (Wilder et al. 2013). Like most arthropod predators, spiders use extra-oral digestion to efficiently extract resources from prey (Cohen 1995). Following nutrient intake and assimilation, the primary nitrogenous excretory product of spiders is guanine (Foelix 2011). However, the mechanisms by

which spider excreta contributes to soil processes and plant growth is not yet well understood.

The overall goal of this study was to test how variation in prey nutrient content affected spiders and the contribution of spiders and prey to soil nutrient dynamics, including soil respiration and subsequent plant growth. Spiders were placed in microcosms and fed either high lipid or high protein crickets. Each microcosm had a soil base with one of three baseline fertilization levels (no added fertilizer, low fertilization or high fertilization) to test if the effect of predators on soil dynamics varies with soil fertilization level. First, we predicted that the nutrient content of prey would affect spider growth, as nutrition affects the growth of other predators (Wilder 2011). We also predicted that the presence of spiders would significantly increase soil respiration and plant growth, especially at the lowest levels of fertilization. Contrary to consumer-driven nutrient recycling prediction of a positive relationship between nutrients excreted with those ingested, we did not expect an effect of prey nutrient content (high lipid versus high protein) on soil respiration or plant growth

Methods

Mesocosms: Top soil taken from depth less than 3 inches was collected in summer 2015 near a field location managed by the Department of Integrative Biology at Oklahoma State University. The grassland soil (1/2 cup) was combined with peat (1 cup), vermiculite (1 cup), sand (1/2 cup), and potting soil (1/2 cup; Expert Gardener Potting Mix) to make a baseline soil that contained natural microorganisms but was very low in baseline nutrient content (i.e. high additions of low nutrient-containing peat, vermiculite, and sand). This soil was used to create a soil nutrient treatment with 3 levels of fertilization. Nutrient additions consisted of combinations of Super Triple Phosphate (45% phosphate, P_2O_5), Ammonium Nitrate (34% nitrogen, N), and potash (60% potash, K_2O) to achieve total content of nitrogen to phosphorus treatments. The soil nutrient levels were 0.0 (0mg nitrate, 0mg phosphate, 417mg potash), 0.03 (82mg nitrate, 5mg phosphate, 417mg potash), and 0.1 (273mg nitrate, 16mg phosphate, 417mg potash). Although soil element quantities varied by treatments, the ratio of elements did not and was held close to typical grassland soil ratio (13:1, Cleveland and Liptzin 2007). The mixture was added to a 16oz (473 mL) plastic cup, enclosed by a 32oz (946 mL) cup (Figure 1). A set of these established mesocosms included spiders (n= 56), while another set did not include spiders (n= 30).

Spiders and Prey: Female black widows (*Latrodectus mactans*) were collected from residences in Stillwater, Oklahoma during 2015. These spiders produced egg sacs in the lab and the spiderlings were reared to sub-adulthood (3rd or 4th instar). The spiders were maintained at constant 25 ± 1^{o}C and 14L:10D light regime in the lab. They were lightly misted with water and fed twice per week on vinegar flies (*Drosophila melanogaster*;

Carolina Biological Supply) and crickets (*Acheta domesticus*; Fluker Farms) of roughly half of each spider's body mass.

Spiders were introduced to mesocosms and acclimated for one week. Eighth-inch sized crickets (*Acheta domesticus*; purchased from Fluker Farms, Louisiana), were maintained on either 10:45:45 or 100:00:00 (protein:lipid:carbohydrate) diets for at least one week before being used as prey (Barnes et al. In Review). The crickets were provided water ad libitum, which was exchanged at least twice weekly. Two crickets were provided to spiders twice per week. The mesocosms were lightly misted twice per week. The total duration of spider presence in the mesocosms was 2 months. At the end of the 2-month feeding period, spiders were killed via freezing and stored in a freezer. We photographed the dead spiders using a Canon Powershot G16 and measured tibia-patella length of the right front legs using ImageJ software (National Institutes of Health). Then, spiders were dried at 60 ^{o}C for 24 hours and weighed. Lipids were measured from a subset of the spiders (n=32 out of 56 spiders) as the difference in mass before and after sequential soaking and extraction in chloroform over the course of three days (Wilder et al. 2013).

Soil Respiration: At the end of the 2-month feeding period, soil was placed in individual 946 mL deli containers, with lids modified to include sealable ports. Respiration was measured as accumulated CO_2 (ppm) using a Li-840A CO_2/H_2O gas analyzer (LI-COR Biosciences) and converted into percent by dividing by 10000. To calculate VCO_2 this was then divided by 100 and multiplied by the volume of the container (946 mL). The metabolic rate was calculated as the end minus start respiration reading, divided by the sampling duration.

Plant Growth: Following measurements of soil respiration, the upper plastic container was removed. The lower plastic containers with soil were then randomly re-arranged within another shelving unit at constant 24± 1 ^{O}C and 10D:14L light regime and an automated water drip system (Claber Spa, Italy) was installed for hydration of each container. The containers were individually supplemented bi-weekly with 0.1mL of potash solution (50g mixed in 200mL of water). Brussels sprouts (*Brassica oleracea var. gemmifera*) seeds were purchased from a commercial distributor (West Coast Seeds). We grew the plants on the soils for 2 ½ months and then we collected the wet and dry mass of the aboveground plant parts (i.e. stem and leaves). These parts were photographed using a Canon Powershot G16. Plant height and leaf area were determined using ImageJ software. We digitally measured plant height and the perimeter around each leaf was traced in the software for measuring the area of each leaf. Then, the areas of all leaves were summed to determine total leaf area.

Statistics: We tested for dietary differences (i.e. protein-biased versus lipid-biased diets) in spider mass, leg-length, and spider percent lipid composition using t-tests. We also tested soil respiration, plant height, quantity of true leaves, true leaf area, true leaf mass, and aboveground plant mass using ANCOVA in JMP 12 software package (SAS Institute, Cary, NC, USA), using predator diet as an ordinal variable and soil treatment as a continuous variable (i.e. covariate). Data for soil respiration, true leaves, true leaf area, true leaf mass, and aboveground plant mass were log-transformed to establish normal distribution. We first used ANCOVA to test if there were differences between the two macronutrient treatments (high protein vs. high lipid prey). If not, we then lumped both

prey types into one “spider present” treatment to test for an overall effect of spider presence vs. absence as a nominal variable against the soil treatment continuous variable When we detected statistical significance level of alpha 0.05 in ANCOVA, we tested for treatment differences of Least Squares Means using post hoc t-tests.

Results

Spider growth: We found significant differences in the mass and leg-length of spiders fed higher lipid versus higher protein crickets. At the end of the feeding period, lipid-fed spiders were significantly heavier than protein-fed spiders ($t_{1,53}$= -3.74, p= 0.0005). Further, lipid-fed spiders developed longer leg lengths (i.e., tibia-patella, which is often used as a measure of fixed body size in web-building spiders) ($t_{1,53}$= -2.24, p= 0.03; Figure 2). Finally, spiders fed high-lipid diets had significantly more lipid in their bodies (mean= 15.2 ± 1.2%) compared to those fed high-protein diets (mean= 8.7 ± 1.0%; $t_{1, 30}$= -4.18, p= 0.0002).

Soil Fertilization and Spiders: Whether spiders were fed high lipid or high protein diets did not influence soil respiration ($F_{1, 44}$= 0.61, p= 0.44), plant height ($F_{1, 44}$= 0.28, p= 0.60), leaf number ($F_{1, 44}$= 1.95, p= 0.17), leaf mass ($F_{1, 44}$= 0.0018, p= 0.97), leaf area ($F_{1, 44}$= 0.08, p= 0.78), and aboveground mass ($F_{1, 44}$= 0.0003, p= 0.99). Soil treatment did not influence soil respiration ($F_{1, 44}$= 0.53, p= 0.47), plant height ($F_{1, 44}$= 0.0003, p= 0.99), leaf number ($F_{1, 44}$= 0.28, p= 0.60), leaf mass ($F_{1, 44}$= 0.01, p= 0.92), leaf area ($F_{1, 43}$= 0.01, p= 0.92), and aboveground mass ($F_{1, 44}$= 0.0066, p= 0.94). The interactive effect of spider diet and soil significantly influenced true leaf mass ($F_{1,44}$= 4.81, p= 0.03), where the high-protein 0.1 soil fertilization group was greater than the high-protein 0.03 soil fertilization group, high-protein 0.0 fertilization group, and the high-lipid 0.1 soil fertilization group. But, we did not find a significant interaction effect on soil respiration ($F_{1, 44}$= 0.12, p= 0.73), plant height ($F_{1, 44}$= 0.80, p= 0.38), number of true leaves ($F_{1,44}$= 3.81, p= 0.06), true leaf area ($F_{1,44}$= 3.20, p= 0.08), and aboveground mass ($F_{1, 44}$= 2.97, p= 0.09).

After finding that spider diet did not influence productivity in soil or plants, we pooled both diet treatments into a "spider present" treatment to test how spiders affected soil and plants relative to controls with no spider. We found that soil respiration significantly increased with soil fertilization level ($F_{1, 67}$= 8.16, p= 0.00057), but not the presence of spiders ($F_{1, 67}$= 3.68, p= 0.06). We found a significant interactive effect of soil fertilization level and spiders on soil respiration ($F_{1, 67}$= 4.51, p= 0.04, Figure 3A), where the soil respiration with spiders present at low soil fertilization was greater than when spiders were absent at low soil fertilization but not at the other soil treatment levels. We found significant effects of soil treatment on leaf number ($F_{1, 67}$= 5.85, p= 0.02), true leaf mass ($F_{1, 67}$= 13.27, p= 0.0005), leaf area ($F_{1, 67}$= 11.23, p= 0.0013) and aboveground plant mass ($F_{1, 67}$= 11.17, p= 0.0014). However, we did not find significant effects of soil treatment on plant height ($F_{1, 67}$= 3.47, p= 0.06). Nor did we find that spider presence significantly influenced plant height ($F_{1, 67}$= 0.27, p= 0.60), leaf number ($F_{1, 67}$= 0.41, p= 0.52), leaf mass ($F_{1, 67}$= 0.48, p= 0.49), leaf area ($F_{1, 67}$= 1.11, p= 0.30), and aboveground plant mass ($F_{1, 67}$= 0.10, p= 0.75). We did not find an interaction effect of soil fertilization treatments and spider presence on plant height ($F_{1, 67}$= 1.14, p= 0.29), number of true leaves ($F_{1, 67}$= 1.73, p= 0.19), true leaf mass ($F_{1, 67}$= 2.88, p= 0.09), true leaf area ($F_{1, 67}$= 2.18, p= 0.14), and aboveground mass ($F_{1, 67}$= 2.37, p= 0.13; Figure 3B).

Discussion

Our results support previous recognition that spiders efficiently extract as many nutrients as possible from prey, even prey that differ in nutrient content, and release a small and constant amount of nutrients to the environment in the excreta and egesta. Specifically, prey nutrient content strongly contributed to predator growth (i.e. higher mass, leg length, and percent lipid on high lipid diets; Figure 2), but had no effect on soil respiration or plant growth. In addition, the presence of spiders overall had very little effect on ecosystem processes, with the only significant effect being soil respiration at the lowest level of soil fertilization (Figures 3A). Thus, while diet is important for the fitness of arthropod consumers the links between arthropod diets, arthropod nutrient deposition, soil productivity, and primary producer growth do not seem to be directly related. Overall, this work combined with previous work (Wilder et al. 2013, Barnes et al. In revision) suggests that spiders assimilate as much of prey biomass as possible regardless of prey nutrient content and leave little behind for decomposer communities. Previous work on herbivores and omnivores has shown that mass balance determines nutrient assimilation versus excretion by consumers (Vanni et al. 2002). Hence, our results suggest that consumer-driven nutrient cycling in a predator, such as spiders, may differ from patterns observed in herbivores and omnivores.

The growth of arthropod carnivores has been suggested to be nitrogen limited, a prediction spurred by the observation that carnivores generally contain more nitrogen in their bodies than herbivores (Fagan et al. 2002). However, the present nutritional state of consumers may not often reflect nutritional demands. That is, the high protein and low lipid (i.e. high C:N) body content of carnivores could be more reflective

of a deficit of lipid than a need for high protein (Wilder and Eubanks 2010). Further, arthropod food webs are increasingly lipid-limited with progressive trophic levels (Wilder et al. 2013). Our findings that body mass, leg length, and lipid stores were higher in spiders fed lipid-biased prey than protein-biased prey support the hypothesis that some predators may be lipid-limited. Recent work on a jumping spider has also shown significantly higher growth of spiders fed higher lipid diets (Wiggins et al. 2018). However, other work has shown that a species of wolf spider grows larger on more protein-biased diet and that the effects of diet on growth differ between wolf spider species (Jensen et al. 2011a, Jensen et al. 2011b). But, the consequences of spider nutrient consumption and deposition upon soil and plant productivity are less recognized.

While prey nutrient content affected predator growth, there was no significant effect of prey nutrient content on soil respiration or plant growth. This is not unexpected, as a recent study found that spiders consume nearly all nutrients and leave the largely indigestible prey exoskeleton (Barnes et al. In Revision). This study found that nutrient consumption by spiders is high across diets ranging from lipid-biased to protein-biased. Further, the high assimilation by spiders meant that the amount of nutrients deposited as prey remains and excreta did not differ by diet. So, the processes of predator consumption and deposition are not likely to be directly linked to plant growth. But, this does not mean that predators cannot influence ecosystem nutrient cycling. For example, Hawlena et al. (2012) found that small deposits of nutrient can prime belowground microbial communities and stimulate decomposition of litter. Large mammalian carnivores can have strong impacts on plant growth and nutrient cycling (Schmitz et al. 2010, Ripple et al. 2014), but these impacts are not yet well investigated in

arthropod systems. Further work is needed to better understand the direct versus indirect effects that predators may have on nutrient cycling, such as linking soil and plant processes.

The soil used in our study was mostly artificial and contained only a small representation of native grassland soil microbial community. The peat, vermiculite, and sand applied to our soil mixture for maintaining moisture content should have been both very low in microbe abundance and have high C:N. We did not find that predator diets affected soil respiration and plant growth, instead only a difference between the presence and absence of spider effects at the lowest fertilization level. However, the study by Hawlena et al. 2012 investigated effects of different prey carcass compositions supplemented to native soils and found that decomposition of leaf litter (i.e. more complex organic matter) was lower with high C:N carcasses. The scope of impacts from nutrient supplementation by aboveground organisms to below ground communities could be dependent on the capacity for microbes priming for the decomposition of organic materials. To more effectively replicate many natural systems (e.g. grasslands) and more strongly test for effects of predators, future studies should use native soils with representative organic matter such as grasses and leaf litter.

Our findings highlight that nutrient content of prey has a larger influence on spiders than it does on how spiders influence nutrient cycling through their egesta and excreta. A key ecological stoichiometry prediction for consumer-driven nutrient recycling is that a positive relationship persists between nutrient intake and waste excreted (Elser and Urabe 1999, Sterner and Elser 2002). However, this broad prediction was developed and largely applied in the context of aquatic and pelagic primary producers and grazers.

More recent research has demonstrated that nutrient recycling rates of aquatic predators are lower than aquatic herbivores (Vanni and MacIntyre 2016), however, mechanisms driving terrestrial carnivore consumer-driven nutrient cycling are not yet well understood. Our results highlight that nutrient recycling links between terrestrial carnivores and plants could predominantly persist as an indirect relationship, requiring consideration of the intermediate soil priming process. Comparative analyses of multiple spider and other terrestrial predator taxonomic groups could be applied to evaluate if this relationship is predominant amongst taxa different from those in which consumer-driven nutrient cycling predictions were developed. A potentially transformative area for future research will be further disentangling the mechanisms of predator intake and recycling within nutritional ecology theory.

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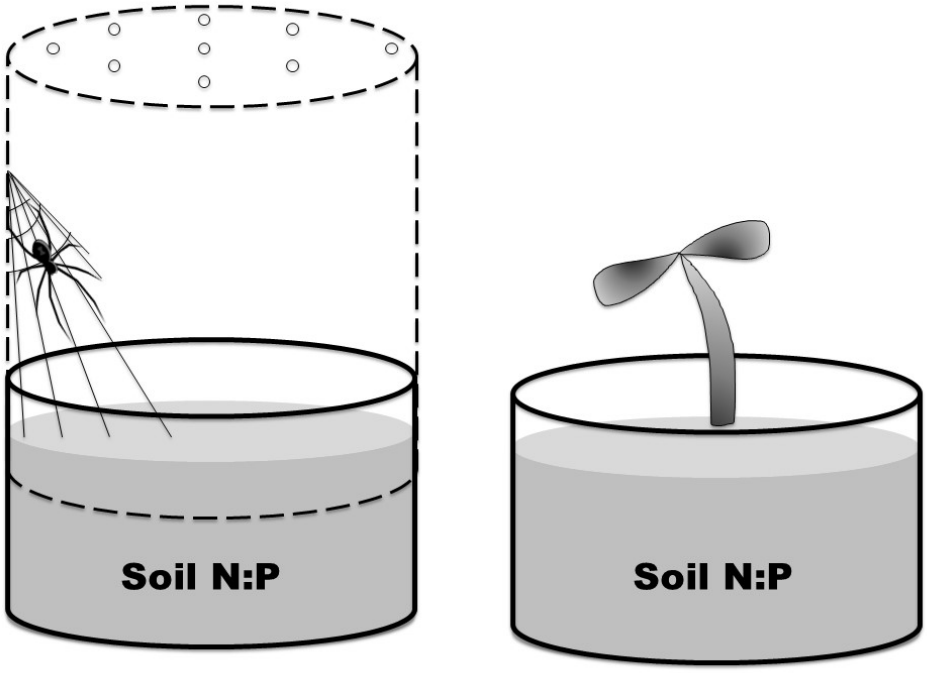

Figure 1. Experimental microcosm setup for duration of black widow and prey carcass resource deposition to soils. A 32oz plastic cup (dashed outline) enclosed spiders, prey, and a 16oz plastic cup base (solid outline) filled with soil (left). Following the duration of nutrient supplementation, soil respiration was measured. Then the clear plastic cup enclosing the soil base was removed and Brussels sprouts were grown on the soils (right).

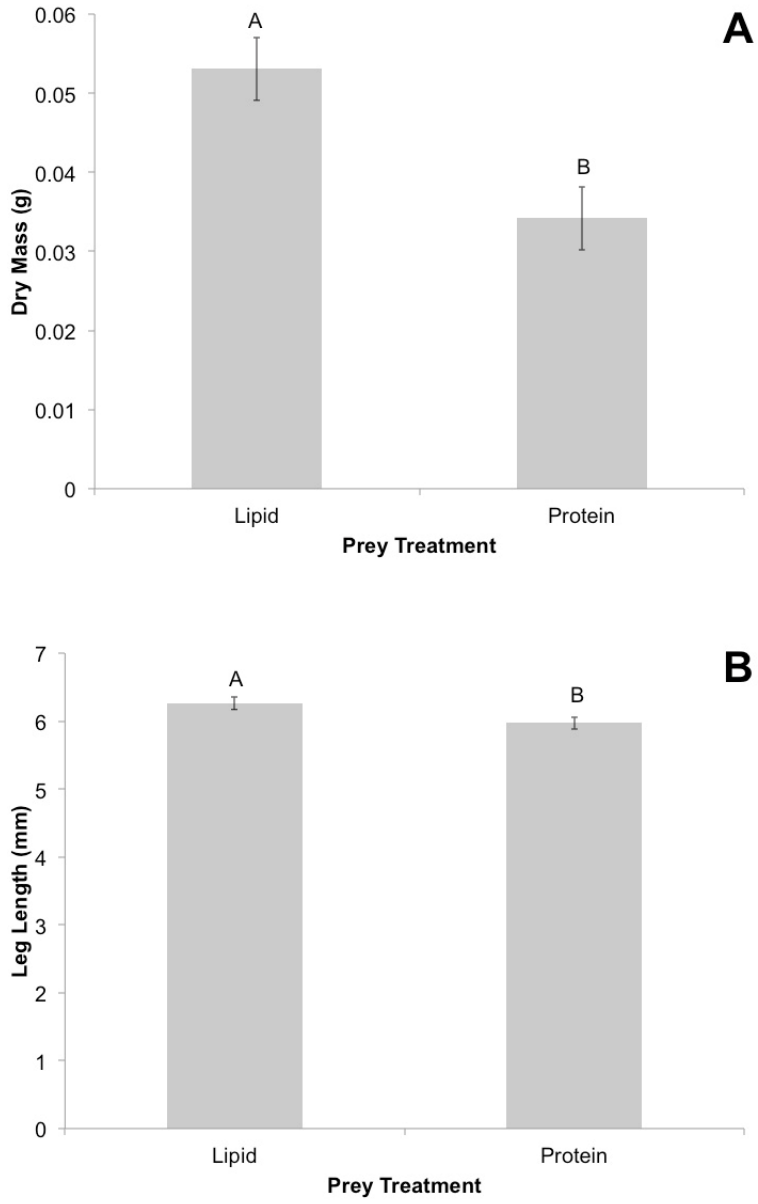

Figure 2. Spider dry mass (A) and leg length (B) on either high lipid or high protein diets. Different letters indicate statistically significant differences between treatments.

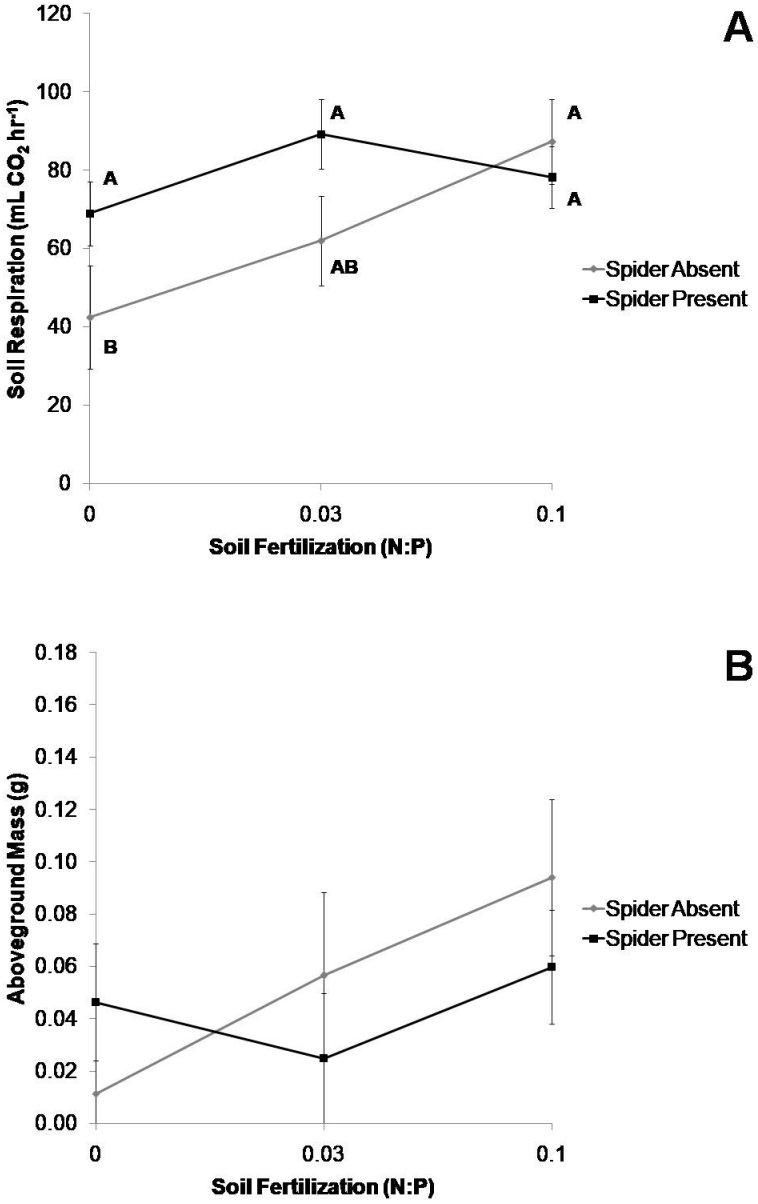

Figure 3. Soil respiration (A) and plant aboveground mass (B) at increments of soil fertilization and presence or absence of spiders. Different letters indicate statistically significant differences between treatments.

CHAPTER IV

Solitary Predators Contribute to Spatial Heterogeneity in Soil Nutrients and plant Growth

Abstract

The roles of predators in nutrient cycling have primarily been considered from trophic cascade perspectives. However, predators also contribute via deposition of prey remains and excrement. These deposited nutrients could have locally strong impacts on soil and plant productivity, especially when predators remain within particular patches of space. Spiders are among the most abundant and diverse predators in many ecosystems. Further, the foraging and nutrient deposition boundaries of web-building spiders are tightly linked to the extent of the web. The goal of this study was to test if the web-building black widow (*Latrodectus mactans*) contributes to the spatial heterogeneity of nutrient distribution, including potential supplementation for soil and plants, using laboratory mesocosms. We observed that spiders generally captured prey within the confines of the web, consumed and excreted prey nutrients near their refuge, then re-deposited the prey carcasses close to the web perimeter. Consequently, we found that soil respiration, plant nutrients, and plant growth was greatest beneath the spider's refuge than at further distances away. However, these patterns do not match the soil nutrients

present, which suggest that spiders promote productivity in soil communities more than by simply supplying nutrients. Instead, we propose that labile spider excreta nutrient deposition effects on plants are mediated by the priming of soil processing of more complex and prevalent nutrients. Given their high abundance within many ecosystems, we propose that spiders are significant contributors to multi-trophic level nutrient cycling via depositional effects.

Introduction

Predators contribute to ecosystem nutrient cycling via consumptive and non-consumptive processes. Ingestion and excretion of nutrients within habitats are examples of consumptive processes (Schmitz et al. 2010). Nutrients can be deposited in the forms of uneaten parts of prey carcasses and excrement. Excrement generally contains forms of nitrogen readily usable by soil microbes (e.g. urea or uric acid). For example, ocean-derived nitrogen in seabird guano subsidizes terrestrial ecosystem nutrient cycling (Fukami et a. 2006) and spider feces contribute significant amounts of nitrogen to bromeliads (Romero et al. 2006). In addition, although predators are often efficient at extracting prey resources (e.g. Barnes et al. In revision), superfluous killing is not uncommon (Maupin and Riechert 2001) and some predators are selective in the part of prey that they consume versus discard (Mayntz et al. 2005). The role of predators in nutrient cycling is relatively well established. Yet, most examples are from systems where there are relatively high densities of or large predators and it remains unclear if solitary and smaller predators can have a significant influence on local nutrient cycling.

Consumer nutrient deposition can shape ecosystem function by increasing variation in the spatial distribution of nutrients. On the North American prairies, bison consume recalcitrant plant biomass but deposit labile nitrogen as dung and urine. This creates patchy hotspots for increased plant nitrogen content and in turn more nutritious forage for the bison (Knapp et al. 1999). Wolves also influence spatial heterogeneity of soil nutrients, soil microbial processes, and plant quality via depositing moose carcasses. At wolf predation sites, there is greater soil inorganic nitrogen (i.e. nitrate and ammonium), phosphorus, and potassium, which contributes to greater leaf nitrogen

content (Bump et al. 2009). Thus, deposition of excreta and carcass nutrients by terrestrial consumers should contribute to spatial heterogeneity of nutrient flow and availability within ecosystems.

Spiders are among the most diverse and abundant terrestrial carnivores. Spiders also consume a large amount of prey worldwide, which has been estimated to be 400-800 million tons per year (Nyffeler and Birkhofer 2017). Like most arthropod predators, spiders feed using extra-oral digestion in which they extract nutrients from prey and discard uneaten parts in the prey carcass (Cohen 1995). Many spiders exhibit significant site fidelity, including building an immovable web, burrow or retreat (Foelix 1996). As such, spiders have the opportunity to deposit excreta and prey carcasses in a localized area for a significant period of time and potentially alter soil nutrient content and plant growth in that area, which could increase spatial heterogeneity in ecosystem processes.

The goal of this study was to test if the presence of spiders altered soil nutrient content and plant growth and contributed to spatial heterogeneity in nutrients, soil respiration, and plant growth. We addressed this goal by maintaining black widow spiders (*Latrodectus mactans*) within laboratory mesocosms in which their webs were confined to one corner of the container. We measured soil nutrients, soil respiration, and plant growth in samples of soil taken under the spider retreat, at the edge of the spider web, and away from the spider web in each mesocosm. Soil nutrient content and plant growth were measured in separate soil samples. We predicted that soils taken from beneath the spiders' webs would have the highest concentrations of nitrogen compounds, soil respiration rates, plant growth and foliar nutrient levels relative to samples taken at the edge of spider webs, and in areas away from the spider webs.

Methods

Soil and Plant Litter Preparation: Soil and leaf litter were collected in August and September 2017 from a field site maintained by the Department of Integrative Biology at Oklahoma State University. The top soil (< 3 inches depth) was filtered of rocks, plants, and arthropods. Oak leaves and grasses were collected from the same location as the soils. We dried the oak leaves and grasses at 60 ºC for 24 hours, ground the leaves using a blender, and cut the grasses to ½ to 1 cm lengths using scissors.

Mesocosms: We used 15L plastic storage containers (42.5cm x 30.2cm x 17.8cm) for the 48 mesocosms (Figure 1 A). Half of the mesocosms were used to test the effects of predators on soil metabolism and nutrient content (n= 24) and the other half were for plant growth (n= 24). In each mesocosm, one corner was abraded to permit spiders to climb and the cap of a 50 mL centrifuge tube was attached in this corner as a shelter. The remaining corners and sides of the container were coated in a thin layer of vaseline to prevent climbing by spiders and prey. The substrate consisted of 3 cups of soil mixed with 3/8 cup of sieved oak leaves and 1/8 cup grasses. Opposite the shelter, a 1-way escape port for the prey was constructed using fencing to alleviate potential prey density-driven effects. The escape port was enclosed with a removable 473 mL plastic deli cup.

Spiders and Prey: Female black widows (*Latrodectus mactans*) were collected from residences in Stillwater, Oklahoma during spring 2017. These spiders produced egg sacs in the lab and the spiderlings were reared in the laboratory. The spiders were maintained at constant 25 ± 1ºC and 14L:10D light regime in the lab. They were lightly misted with water and fed twice per week on vinegar flies (*Drosophila melanogaster*) and crickets

(*Acheta domesticus*) of roughly half of each spider's body mass. Spiders were raised to the fourth instar for experiments, which is past the minimum size required to differentiate between sexes. Only sub-adult female spiders were used in experiments because males feed irregularly and adult females continue growing for a longer time and to a larger size than males.

Spiders were individually assigned to each of the 48 mesocosms and acclimated for one week. Three, one-week old crickets (*Acheta domesticus*; purchased from Armstrong's Cricket Farm) were placed into each mesocosm twice per week. Prey were placed in an area of the mesocosm that did not have web to allow for more natural predator-prey encounters. The mesocosms were lightly misted twice per week and any parts of the spider webs extending beyond the abraded corner boundaries were trimmed. Crickets in the escape port were tallied and removed twice per week. Very few of the crickets placed in the mesocosms were found in the escape port (2.4±0.2% of total crickets). The total duration of the experiment was 3 months.

After we removed the spiders from the mesocosms, we collected soils from the corner directly under the spider retreat, the center of the mesocosm, and the corner opposite from the spider corner (Figure 1A). To remove soil, an alcohol-sterilized compass (Xacto Precision Compass Cutter; Elmer's Products, Inc.) was used to draw a line in the soil that was 15 cm radius (i.e., a 90^{o} arc with the compass centered in the corner of the container) from the corner for the spider retreat and opposite corners and a 7.5 cm radius circle from the mesocosm center. These sections of soil were removed and placed into individual plastic cups. The volume of each soil core was approximately ½ cup.

Soil Measurements: Twenty-four of the mesocosm replicates were randomly selected to destructively measure soil nutrient content (i.e., these containers did not receive plants). Prior to nutrient measurements, we used closed-system respirometry to measure soil respiration (e.g., carbon mineralization) of each core for 24 hours. Soil cores were placed in individual 946 mL deli containers, with lids modified to include sealable ports. Respiration was measured as accumulated CO_2 (ppm) using a Li-840A CO_2/H_2O gas analyzer (LI-COR Biosciences) and converted into percent by dividing by 10000. To calculate VCO_2 this was then divided by 100 and multiplied by the volume of the container (946 mL). The metabolic rate was calculated as the end minus start respiration reading, divided by the sampling duration and mass after being dried at 60 ^oC for 24 hours.

Finally, ammonium, nitrate, total nitrogen, and phosphorus in twelve randomly selected mesocosms was measured by the Oklahoma State University Soil, Water, and Forage Analytical Laboratory. Ammonium and nitrate were extracted using 1M KCl. Ammonium was analyzed using the salicylate method and nitrate was analyzed using the cadmium reduction method, then measured using a Lachat flow injection autoanalyzer (Hach Company). Phosphorus was extracted with Mehlich 3 and analyzed with a Spectro ICP Spectrometers (SPECTRO Analytical Instruments). Post hoc power analyses at the alpha significance level of 0.05 suggested that chemical analysis of the remaining mesocosms would not change the outcome of the statistical results.

Plant Growth: A separate set of twenty-four mesocosms (i.e., those not used for soil measurements) was used to test the effects of the distance from the spider retreat on plant growth. Soil cores used for plant growth were taken following the same protocol as those for soil analyses. These soil cores were transferred to 473 mL (16 oz) plastic deli containers, lightly misted, and provided continuous water using a cotton wick that extended down into a cup of water.

Brussels sprouts (*Brassica oleracea var. gemmifera*) seeds were purchased from a commercial distributor (West Coast Seeds). Brussels sprouts seeds were sprouted on moist paper towels for 3-4 days prior to planting in soil. Plants were allowed to grow on the soils for 2 ½ months and then we collected the aboveground plant parts (i.e. stem and leaves). These parts were photographed using a Canon Powershot G16 at 5x zoom mounted on a tripod. Plant height and leaf area were determined using ImageJ (National Institutes of Health) software. The perimeter around each leaf was traced in the software and then the areas of all leaves were summed to determine total leaf area. The aboveground biomass was also weighed wet, all leaves were dried and weighed, and the nutrient content of leaves was measured.

After measuring the dry mass, we ground and then subsampled the leaves using elemental and macronutrient analyses. We measured the carbon and nitrogen content with an elemental analyzer (Elementar Americas, Inc., Mt. Laurel, New Jersey). We also measured both leaf protein and total non-structural carbohydrate content, but not lipids as they are typically present in very low concentration in leaves (Davis et al. 2004). The protein content of dried, ground leaves were determined in triplicates using the Bradford

Assay (Wilder et al. 2013). We measured non-structural carbohydrate using the Phenol-Sulfuric Acid Assay (Albalasmeh et al. 2013).

Statistics: Mesocosm location differences in soil mass, soil nutrient content, plant growth, plant nutrient content, and leaf area were examined using ANOVA. Soil respiration was examined using ANCOVA, using soil dry mass as a covariate. We applied ANOVA and ANCOVA using JMP 12 software package (SAS Institute, Cary, NC, USA). When we detected statistical significance in ANOVA, we tested for treatment differences of Least Squares Means using Tukey post hoc tests.

Results

Spiders, Soil, and Distance from Web: During observations of spiders (i.e., when watering and feeding spiders) most spiders were located and observed feeding on prey close to their retreat. The webs of spiders often extended into the center of the mesocosms. We observed that spiders generally captured prey near the soil surface, briefly wrapped the prey in silk, and then dragged the prey up to the retreat (Figure 1B). Within the retreat, the spiders typically spent several hours digesting the prey before discarding prey remains resembling a hollow, but otherwise intact, carcass. Spider excrement generally appeared below the retreat, but often the prey carcasses were deposited closer to the edge of the spider web, which was near the center of the mesocosm.

Soil nitrate (Figure 2A; $F_{2,33}= 2.57$, $p= 0.09$) and total nitrogen (Figure 2C; $F_{2,33}= 0.40$, $p= 0.68$) did not differ by location (i.e., below spider, center, or away from spider). But, soil ammonium (Figure 2B; $F_{2,33}= 3.64$, $p= 0.04$) and total phosphorus (Figure 2D; $F_{2,33}= 4.76$, $p= 0.02$) were significantly higher in the center of the mesocosm compared to the corner away from the spider. Soil ammonium and total phosphorus in the sample below the spider web was intermediate and not significantly different from the samples in the center and away from the spider.

For soil respiration, there was a significant effect of soil location with higher respiration under the spider retreat than in soil from the center or away from the spider web (Figure 3; $F_{2,69}= 5.25$, $p= 0.0077$). There was significant difference in soil dry mass ($F_{2, 69}= 0.61$, $p= 0.55$) and no effect of soil mass on respiration ($F_{1,70}= 0.67$, $p= 0.41$).

Further, there were no differences in the masses of soil cores between locations ($F_{2,69}$= 0.61, p= 0.55). Nor was there an interaction between location and dry mass on soil respiration ($F_{2,69}$= 0.70, p= 0.50).

Plant Growth: Brussels sprouts grown on soils collected from beneath spiders grew significantly larger than those grown on soils taken from the corner away from the spider (Table 1, Figure 4). Plants grown on soil from the center were intermediate between those under the spider retreat and away from the spider. Plant aboveground wet mass ($F_{2,69}$= 6.68, p= 0.0022), true leaf wet mass ($F_{2,69}$= 6.44, p= 0.0027), and true leaf dry mass ($F_{2,69}$= 5.72, p= 0.0050) were all greater in plants grown on soils taken from below spiders than further away. Further, plants grown on soils beneath spiders had a significantly higher number of true leaves ($F_{2,69}$= 6.27, p= 0.0031) and true leaf area ($F_{2,69}$= 7.44, p= 0.0012). Plant height did not differ by distance from the spiders ($F_{2,69}$= 1.30, p= 0.28).

Plant Composition: We compared the nutrient content of plants in two ways: first as the total amount of nutrients in plants (Table 1), which takes into account plant size; and then as the concentration of nutrients in plant tissue (Table 2), which is independent of plant size. As expected given the greater true leaf mass of the plants grown on soil beneath the spiders, the total amount of carbon, nitrogen, carbohydrate and protein in plants were significantly higher in plants grown in soil under spider webs compared to plants grown on soil located away from spiders, with intermediate values in the center of the mesocosms (Table 1). In terms of nutrient concentrations in plant tissue (i.e., independent of plant size), carbon ($F_{2,67}$= 4.90, p= 0.01) and carbohydrate ($F_{2,69}$= 5.57, p= 0.0057) concentration of plant leaves were greater in plants grown beneath spiders compared to

plants grown away from spiders. However, nitrogen ($F_{2,67}$= 0.02, p= 0.98) and protein ($F_{2,60}$= 0.06, p= 0.95) concentrations did not differ with distance from spiders.

Discussion

Our results support the hypothesis that a small, solitary predator can have a significant effect on the spatial distribution of soil nutrient content, soil respiration and plant growth. Plant mass and total nutrient content (Tables 1-2, Figure 4) were highest under spider retreats and declined with distance away from the retreat. Soil respiration was also highest directly under the spider retreat (Figure 3). Yet, common nutrients important for plant growth were highest at the edge of the spider web and not significantly different between locations under the spider retreat and away from the spider (Figure 2). Hence, while the presence of spiders significantly benefits plant growth, this effect does not appear to be solely driven by common soil nutrients as the patterns of soil nutrients and plant growth were not the same.

There are several potential explanations for the different patterns of soil nutrients and plant growth. First, the primary excretory product of spiders is guanine (Foelix 1996). We did not measure guanine. It is possible that guanine was directly used by plants without being converted to other soil nutrients (e.g., ammonia, nitrate, etc.). Secondly, spider excreta could increase microbial decomposition processes in ways that benefit plants, either due to nutrient input in general, guanine specifically, or other aspects of the excreta (e.g., gut microbes). In another study, a small shift in the nutrient content of grasshopper carcasses (i.e., due to fear of predation by spiders) resulted in large changes in soil carbon mineralization potentially by priming microbial communities (Hawlena et al. 2012). Further research of the contribution of predator nutrient deposition in soil microbial communities and biogeochemical pathways could offer greater insight into aboveground-belowground feedbacks and broader ecosystem nutrient cycling.

Predators deposit nutrients in several forms. Our observations suggested that excreta were primarily deposited under the spider retreat while carcasses were often discarded towards the edge of the web. Excreta generally provides soil with labile forms of carbon and nitrogen. Compared to excrement, uneaten parts of prey carcasses generally may be of lower quality because they primarily consist of skeleton, exoskeleton, or other indigestible molecules. For example, arthropod exoskeleton can take years to decompose in nature (Seastedt and Tate 1981). Yet, carcasses could also contain labile nutrients (e.g., remains of soft tissue that were difficult to extract) as evidenced by the higher ammonium and phosphorus in soil under the edge of the spider web. Hence, spiders contribute to variation in ecosystem processes at two scales: areas with spiders have higher soil fertility and plant growth than areas without, and within a spider web (i.e., retreat versus edge) there appear to be differences in the types of nutrients deposited and their effects on soil nutrients and respiration.

In our experiment, plants grown on soils beneath spiders clearly incorporated more nutrients and grew larger than those away from spider webs (Tables 1-2, Figure 5). Plants developed greater concentrations of carbon and carbohydrates (Table 2). Our experimental findings indicate that not only do spiders supplement the soil surface with nitrogen, but also that spiders might stimulate decomposition in the litter (e.g. oak leaves, grasses, and interstitial soil organic matter). Further, approximately twice as much non-structural carbohydrates and protein would potentially be available for a plant-consumer when spiders are present (Table 1). Predator contribution to soil nutrient cycling could therefor introduce indirect, positive feedback on herbivore quality via nutrient deposition and promotion of soil mineralization.

Our result support the hypothesis that plants and spiders can be mutualist partners (Romero et al. 2005, 2006). Plants provide shelter for spiders (e.g., locations for retreats, attachment points for webs, cover from sunlight) and spiders provide plants with nutrients from prey and protection from herbivores. In addition to our results on web-building black widow spiders, other studies of jumping spiders have shown that they use plants for structure and in return contribute substantial quantities of nitrogen in both excrement and prey remains (Romero et al. 2005, 2006). Predatory dipteran larvae can also contribute to nutrient supplementation in pitcher plants (Lam et al. 2018). While studies of plant-predator mutualisms often focus on coevolved relationships (e.g., food for protection mutualisms between ants and plants; Wackers et al. 2005), there may be many situations where predators and plants engage in facultative, non-coevolved mutualisms and these mutualisms can have important consequences for ecosystem processes and plant growth.

Spider species vary in their foraging strategies and site fidelity. Solitary web-building spiders have high foraging site fidelity within a localized area, while wandering spiders may range more widely (Foelix 1996). Previous research has shown that the foraging mode of spiders (i.e., active versus sedentary wandering spiders) can have significant effects on plant community composition and ecosystem processes, driven primarily through indirect effects on prey behavior (e.g., fear of predation; Schmitz 2008, Schmitz et al. 2017). It is also possible that predator foraging mode can have direct effects on the spatial distribution of plants and ecosystem processes. Whereas solitary web-building spiders likely concentrate nutrients in particular locations and create a more heterogeneous nutrient landscape, wandering spiders and other predators foraging more

widely or densely could instead moderate spatial variability of nutrient supply across landscapes by spreading prey nutrients throughout the landscape. Greater research of nutrient deposition will be necessary to further understand links between predators, soil nutrients, and plant growth across space.

Our experiments were designed to test the direct effects of spiders on the spatial distribution of soil nutrients and their effects on plant growth. However, in nature, the situation can be more complex as direct and indirect effects of predators on herbivores can combine to influence the overall effect of predators on ecosystem processes (Schmitz 2008, Schmitz et al. 2010). In addition, predators can influence the abundance and activity of detritivores and, hence, their impact on nutrient cycling (Sitvarin and Rypstra 2014, Hawlena and Zaguri 2016). These interactions between predators, herbivores, and detritivores could also depend on the traits of each (e.g., foraging mode, metabolic rate, activity level, etc.), which could lead to context-dependency in the outcome of interactions. Further work examining patterns in the field and the potential mechanisms responsible will be valuable for gaining a greater understanding of the contribution of predators to ecosystem processes.

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Table 1. Plant growth variables and total masses of nutrients present in (mean ± SE) for Brussels sprouts taken from the corner away from the spider, in the center of the mesocosm, and from beneath the spider, respectively.

Plant Variable	Away	Center	Spider	F	P
Height (cm)	4.38±0.22	4.46±0.22	3.98±0.22	1.30	0.28
Number of True Leaves	$4.38\pm0.36_{A}$	$5.45\pm0.36_{AB}$	$6.17\pm0.36_{B}$	6.27	**0.0031**
True Leaf Dry Mass (g)	$0.09\pm0.02_{A}$	$0.16\pm0.02_{AB}$	$0.21\pm0.02_{B}$	5.72	**0.0050**
Carbon Mass (g)	$0.037\pm0.010_{A}$	$0.066\pm0.010_{AB}$	$0.086\pm0.010_{B}$	5.74	**0.0049**
Nitrogen Mass (g)	$0.002\pm0.001_{A}$	$0.003\pm0.001_{AB}$	$0.004\pm0.001_{B}$	5.13	**0.01**
Carbohydrate Mass (g)	$0.028\pm0.011_{A}$	$0.058\pm0.011_{AB}$	$0.090\pm0.011_{B}$	7.43	**0.0012**
Protein Mass (g)	$0.012\pm0.002_{A}$	$0.017\pm0.002_{AB}$	$0.021\pm0.002_{B}$	3.91	**0.03**

Note: Significant differences between locations (F-test) are shown in bold.

Table 2. Concentrations of nutrients (mean mg $100mg^{-1}$ ± SE) in the true leaves of Brussels sprouts from the corner away from the spider, in the center of the mesocosm, and from beneath the spider, respectively.

Nutrient	Away	Center	Spider	F	P
Carbon	$41.06\pm0.18_{A}$	$41.41\pm0.18_{AB}$	$41.86\pm0.18_{B}$	4.90	**0.0103**
Nitrogen	2.11±0.16	2.06±0.16	2.10±0.16	0.02	0.9765
Non-Structural Carbohydrates	$31.10\pm2.29_{A}$	$34.60\pm2.29_{AB}$	$41.70\pm2.29_{B}$	5.57	**0.0057**
Protein	12.46±1.28	12.92±1.38	12.30±1.28	0.06	0.9456

Note: Significant differences between locations (F-test) are shown in bold.

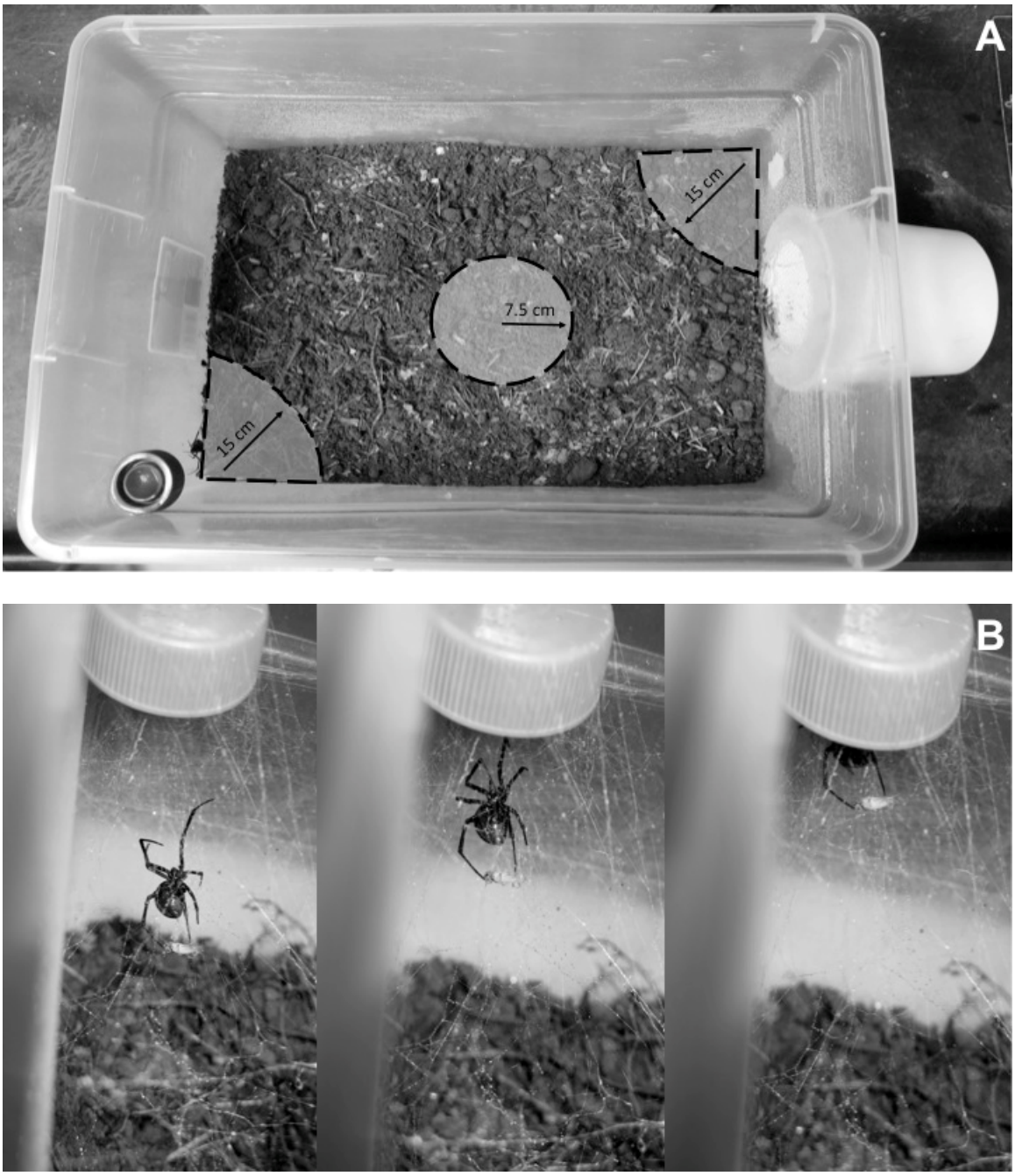

Figure 1. Mesocosm experimental setup for duration of black widow resource deposition to soils (A) and sequence of typical spider feeding event (B). Location of soil cores collected from the corner beneath the spider, the center of the mesocosm, and the corner away from the spider are indicated by the gray regions within the dashed boundary lines. Sequence of spider feeding progressed from wrapping to extra-oral digestion of prey (left

to right). Briefly, spiders envenomed prey near the soil surface, rapidly wrapped the prey in silk, and then dragged the prey up toward the retreat for extra-oral digestion. Post-digested prey were deposited several hours later more closely to the mesocosm center.

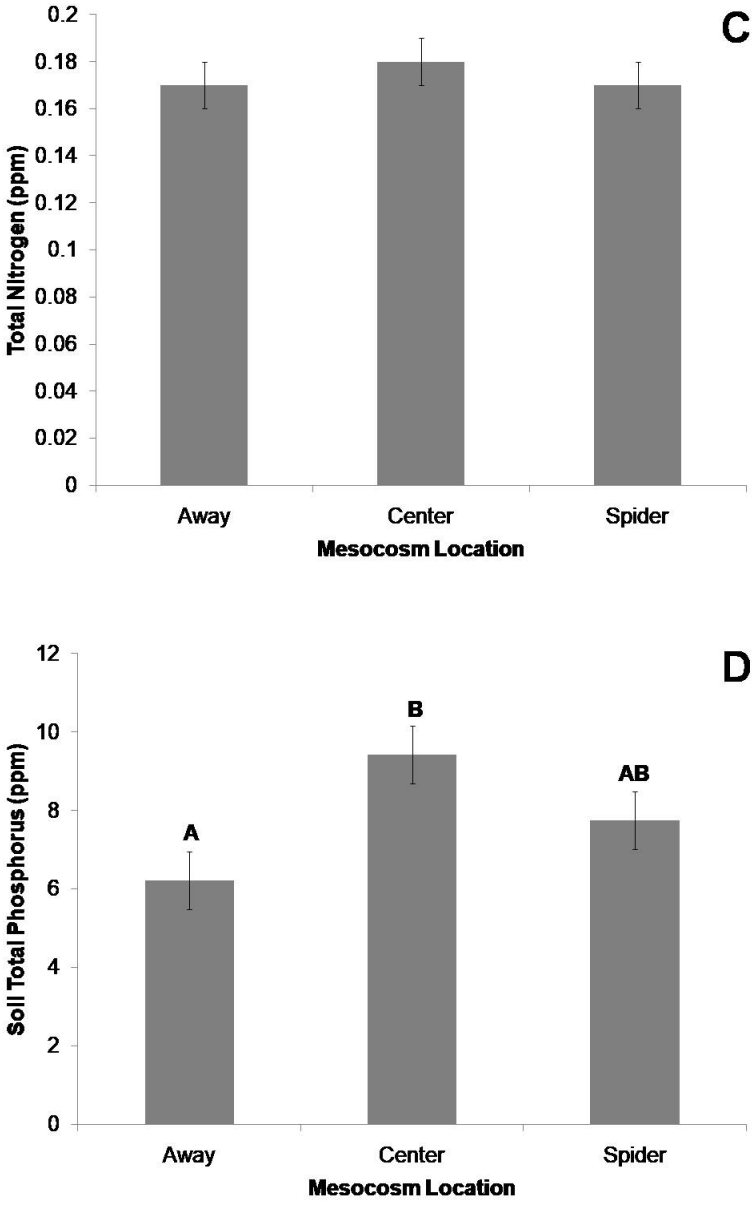

Figure 2. Nitrate (A), ammonium (B), total nitrogen (C), and phosphorus (D) content of soils taken from the corner away from the spider, in the center of the mesocosm, and from beneath the spider, respectively. Different letters indicate significant variation between treatments.

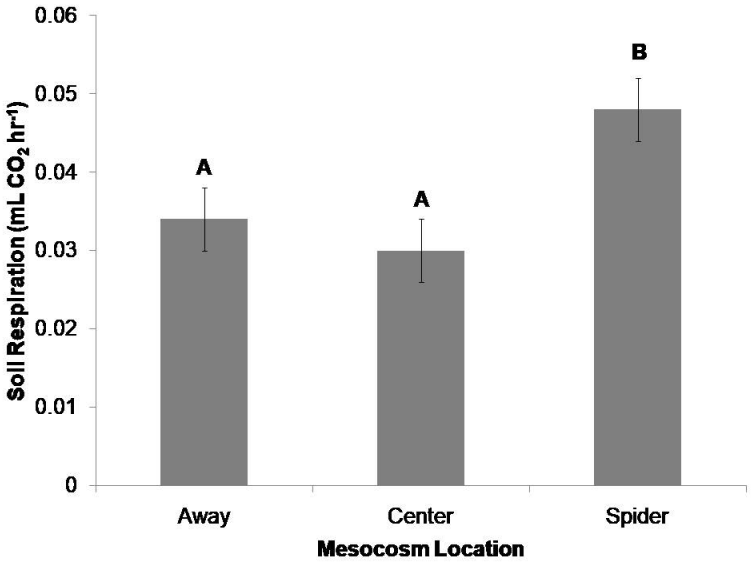

Figure 3. Carbon mineralization (measured through CO_2 production) of soils taken from the corner away from the spider, in the center of the mesocosm, and from beneath the spider, respectively. Different letters indicate significant variation between treatments.

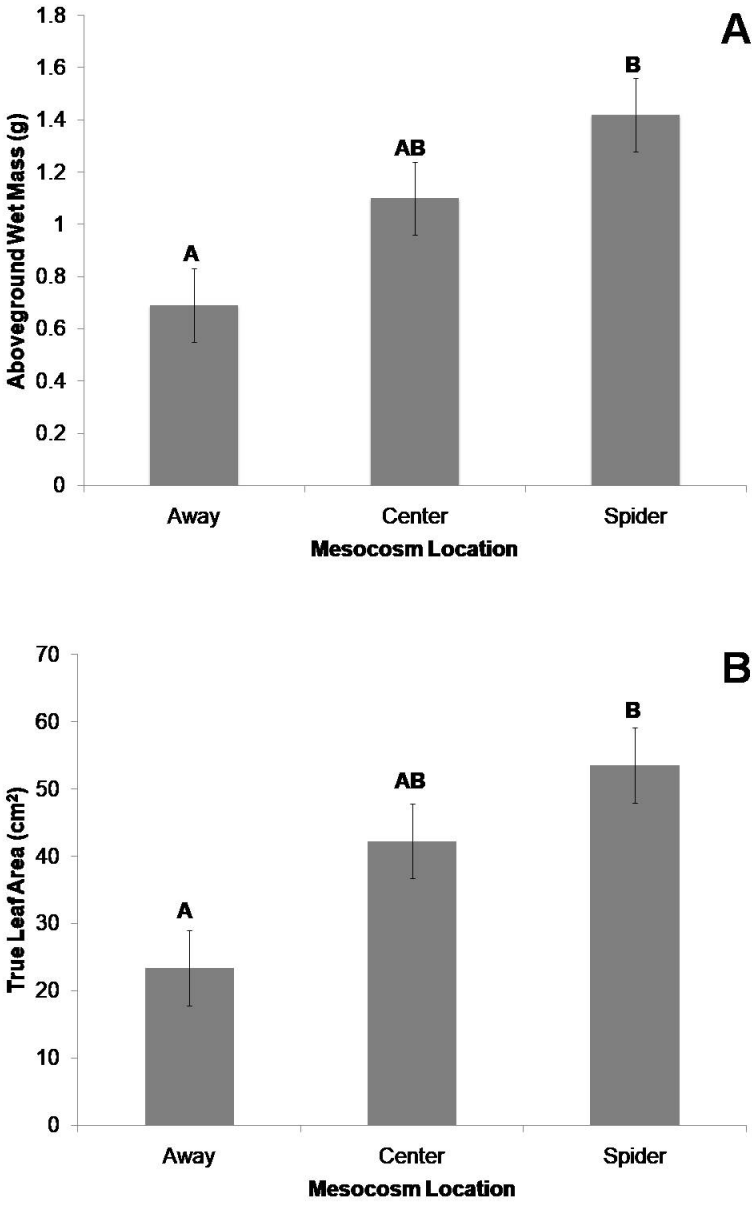

Figure 4. Aboveground wet mass (A) and true leaf area (B) mass of plants grown on soils taken from the corner away from the spider, in the center of the mesocosm, and from beneath the spider, respectively. Different letters indicate significant variation between treatments.

General Conclusions

Predators are important contributors to ecosystem nutrient cycling. Predators can influence nutrient flow within ecosystems via trophic cascades, nutrient consumption, nutrient translocation, and indirect effects of predation (Schmitz et al. 2010). Arthropod predators, such as spiders, can serve particularly impactful functions as biological controls (Symondson et al. 2002). Further, the annual prey consumption by spiders alone has been estimated to feed on an estimated 400-800 million tons of prey per year, which also likely translates into a significant amount of nutrients deposited from these prey (Nyffeler and Birkhofer 2017)

Arthropod prey vary widely in availability (Wise 2006), nutrients (Wilder et al. 2013), and exoskeleton content (Lease and Wolf 2010). Ecological stoichiometry traces the availability and balance of elements through ecosystems (Sterner and Elser 2002). More specifically, ecological stoichiometry links the availability of the approximately 20 biologically relevant elements to organisms' homeostasis. That is, the amount of nutrients assimilated and excreted is predicted to be a function of the amount of nutrients ingested and consumer's body condition (i.e. consumer-driven nutrient cycling; Elser and Urabe 1999, Vanni et al. 2002, Hood et al. 2005). However, support for these predictions using food-limited spiders investigated herein was not clearly demonstrated. Spiders did not differentially deposit nutrients as excreta when fed either lipid-biased or protein-biased prey, despite ingesting and assimilating different amounts of nutrients. Spiders are

generally food-limited in nature, which is in contrast to ad libitum treatments possible within laboratory testing of consumer-driven nutrient cycling, so nutrient consumption and assimilation by spiders could be driven more towards maximization rather than optimization (reviewed in Wise 1993, 2006).

Nutrient consumption strongly shapes the physiology of carnivores. A previous study has found that wolf spiders grow larger on protein diets (Jensen et al. 2011), while another study suggests that other spiders grow larger on lipids or supplement for deficiencies in either nutrients by consuming carbohydrates (Wiggins et al. 2018). In my experiments, both wolf spiders and black widows assimilated more lipid on high-lipid diets than low-lipid diets. Black widows also developed longer leg lengths (i.e., tibia-patella, which is often used as a measure of fixed body size in web-building spiders) and higher lipid composition. Further, both species ingested most macronutrients available within prey, discarded the prey exoskeleton, and deposited very small quantities of excreta. These findings corroborate previous research that lipid becomes more limited with progressive trophic levels (Wilder et al. 2013). However, these findings do not necessarily support the stoichiometric prediction that spiders should differentially recycle nutrients or the prediction that spiders are nitrogen and protein limited (Fagan et al. 2002, Dennno and Fagan 2003).

Although nitrogen is an important component of proteins, my experiments demonstrate the pitfalls of applying universal nitrogen to protein conversion factors for

attempting to understand nutrient intake. That is, nitrogen embedded within the arthropod chitinous exoskeleton is not available for most consumers and to presume otherwise would be an overestimation of protein content (Wilder and Eubanks 2010). The protein-chitin matrix and other structural components within the arthropod endocuticle and exocuticle are potentially indigestible sources of nitrogen, which could inflate estimates of digestible protein if relying on elemental analyses alone (Finke 2007). Further, spiders were less efficient at extracting digestible nutrients from prey containing high amounts of indigestible material (e.g. chitin). However, neither spider species deposited different amounts of nitrogenous excrement with differences in prey macronutrients or exoskeleton. Contrary to the substantial amount of literature suggesting that the magnitude of digestive metabolism (i.e. SDA) should increase with dietary exoskeleton composition (reviewed in Joblin 1983, McCue 2006, and Secor 2009), I did not find this to be the case with black widows. But, the relative proportion of the digestive metabolism to the prey energetic content (i.e. SDA coefficient) was higher and could impose constraints on nutrient intake and allocation. Overall, the findings of my experiments identify the assimilative and metabolic costs for spiders to achieve nutritional needs from differentially digestible prey while highlighting the need for a multi-nutrient approach to consumer-driven nutrient cycling (e.g. relating nitrogen and protein).

While nutrient intake differences are clearly critical for spider physiology, the translation from consumption to deposition to differences in broader ecosystem function is not yet well established. Nutrient deposition is a key link between aboveground consumers, belowground decomposers, and aboveground primary producers. Contrary to the aforementioned consumer-driven nutrient cycling predictions, neither wolf spiders nor black widows in my experiments differed in excrement deposition. Although black widows in the third chapter grew larger on high-lipid diets than on high-protein diets, soil respiration and plant growth did not differ. A previous study found that soil decomposition declines with increased C:N supplementation (Hawlena et al. 2012), however, in this study a much higher input of microbes were available in the predominantly natural grassland compared to our mostly artificial soil with low grassland soil supplementation. Further, in the fourth chapter total soil nutrients did not differ. This finding further suggests that the primary link between spiders and plants is not a direct one, but rather spider nutrient deposition functions to activate soil nutrient recycling processes (Hawlena et al. 2012). Simple, labile nutrients (e.g. spider predation remains and excreta) could be essential for priming the soil microbial community decomposition of more complex organic nutrients at local scales.

The magnitude of nutrients deposited to ecosystems is important, but also important is the location. Localized deposition of nutrients can create hotspots for soil and plant productivity. Seabird excretion of guano on nutrient-poor islands is a critical

input of nitrogen and phosphorus for soil and plant communities (Anderson and Polis 1999). The webs of colonizing spiders are also important for early stages of ecological succession (Hodkinson et al. 2001). Further, jumping spider excrement provides substantial amounts nitrogen to bromeliad plants growing on low-nutrient soils (Romero et al. 2006). In the fourth dissertation chapter outlined here, the spatial location of black widow spiders greatly increased heterogeneity in soil respiration and plant nutrients. I observed a discernible decline in soil respiration, plant nutrient content, and plant growth at distances further from the spider web. Although the spiders had high nutrient intake and assimilation from prey, my experiment demonstrated that even relatively low amounts of nutrients in excreta contributed by a solitary spider might have significant implications for spatial distribution of nutrients within ecosystems. Further, this experiment helps advance our understanding of predator contribution to localized differences in soil and plant productivity.

My results discern the nutrient cycling roles of spiders as opportunistic, generalist predators and predominantly indirect contributors to soil and plant processes. Future study will be necessary for further elucidating the role of terrestrial predators in consumer-driven nutrient cycling. Research areas that remain to be addressed are disentangling the predator intake relationships between the numerous biologically relevant elements and macronutrients, the mechanisms by which individuals' metabolism shapes the fates of nutrients, if predators occupying different trophic positions vary in

contribution to consumer-driven nutrient cycling, and whether specific microbial groups differently influence the nutrients indirectly supplemented by spiders to plants. Finally, inclusion of predators in future development of consumer-driven nutrient cycling perspectives offers a broad opportunity further advance the understanding of ecosystem nutrient flow and function.

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